No. 2015-1123

IN THE

United States Court of Appeals

FOR THE FEDERAL CIRCUIT

ILLUMINA CAMBRIDGE LTD.,
Appellant,

v.

INTELLIGENT BIO-SYSTEMS, INC.,
Appellee.

APPEAL FROM THE UNITED STATES PATENT AND TRADEMARK OFFICE PATENT TRIAL AND APPEAL BOARD IN NO. IPR2013-00128

BRIEF OF PATENT OWNER-APPELLANT ILLUMINA CAMBRIDGE LTD.

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March 10, 2015

CERTIFICATE OF INTEREST

Counsel for Appellant certifies the following:

1. The full name of every party or amicus represented by me is:

Illumina Cambridge Ltd.

2. The name of the real party in interest represented by me is:

Illumina Cambridge Ltd.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curie represented by me are:

Illumina Cambridge Ltd. is a wholly owned subsidiary of Illumina, Inc., a Delaware corporation with its principal place of business in San Diego, CA.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court are:

Knobbe, Martens, Olson & Bear, LLP: Brenton R. Babcock, William R. Zimmerman, Jonathan E. Bachand, Kerry S. Taylor, Nathanael R. Luman, Andrew E. Morrell; Reinhart Boerner Van Dueren s.c.: James G. Morrow, James D. Borchardt.

Dated: March 10, 2015

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OTHER AUTHORITIES

STATEMENT OF RELATED CASES

Pursuant to Federal Circuit Rule 47.5, Appellant provides the following statement of related cases:

(a) This is an appeal of the Final Written Decision from the United States Patent and Trademark Office Patent Trial and Appeal Board ("Board") in *inter partes* review ("IPR") 2013-00128 regarding U.S. Patent No. 7,057,026 ("the '026 patent"), owned by Appellant Illumina Cambridge Ltd. ("Illumina"). No other appeal in or from the proceeding below was previously before this or any other appellate court.

(b) Pending before this Court is appeal No. 2015-1243, an appeal of the Final Written Decision from the Board in IPR2013-00266 regarding U.S. Patent No. 8,158,346 ("the '346 patent"), also owned by Illumina. The '346 patent claims priority to the '026 patent, and the specifications of the two patents largely overlap. On December 5, 2014, Illumina filed an unopposed motion requesting coordination of appeal Nos. 2015-1123 and 2015-1243. The Court granted Illumina's motion by Order dated December 31, 2014.

Additionally, Illumina has alleged infringement of, *inter alia*, the '026 and '346 patents in counterclaims against Appellee Intelligent Bio-Systems, Inc. ("IBS") before the District of Delaware in *Trustees of Columbia Univ. v. Illumina, Inc.*, No. 12-cv-376 (D. Del.). The district court litigation is presently

stayed, except for limited fact discovery, pending the outcome of this appeal and appeal No. 2015-1243, as well as the outcome of three coordinated appeals and an additional *inter partes* review:

- Federal Circuit Appeal No. 2014-1547 (appeal of IPR2012-00006 regarding U.S. Patent No. 7,713,698 ("the '698 patent"), where the Trustees of Columbia University ("Columbia") is the Appellant-patent owner and Illumina is the Appellee-petitioner). IBS is the exclusive licensee of '698 patent;
- Federal Circuit Appeal No. 2014-1548 (appeal of IPR2012-00007 regarding U.S. Patent No. 7,790,869 ("the '869 patent"), where Columbia is the Appellant-patent owner and Illumina is the Appellee-petitioner). IBS is the exclusive licensee of '869 patent;
- Federal Circuit Appeal No. 2014-1550 (appeal of IPR2013-00011 regarding U.S. Patent No. 8,088,575 ("the '575 patent"), where Columbia is the Appellant-patent owner and Illumina is the Appellee-petitioner). IBS is the exclusive licensee of the '575 patent; and
- IPR2013-00517 regarding U.S. Patent No. 7,566,537, where Illumina is the patent owner and IBS is the petitioner. On February 11, 2015, the Board issued a Final Written Decision upholding the patentability of the challenged claims of the '537 patent.

JURISDICTIONAL STATEMENT

On July 25, 2014, the Board issued its Final Written Decision in IPR2013-00128. JA26-57. Illumina timely filed its notice of appeal on September 24, 2014. *See* 35 U.S.C. § 142; 37 C.F.R. § 90.3(a). This Court has jurisdiction pursuant to 35 U.S.C. § 141(c) and 28 U.S.C. § 1295(a)(4)(A).

I. STATEMENT OF THE ISSUE

Did the Board err in denying Illumina's Motion to Amend to add substitute Claims 9-12 to U.S. Patent No. 7,057,026 ("the '026 patent") by:

(1) improperly focusing on a single limitation added by amendment, the disulfide linkage limitation, rather than the combination of amended limitations, specifically a disulfide linkage connecting a label to the base and a 3'-protecting group that are cleavable under identical conditions;

(2) failing to provide any motivation for, or reasonable expectation of success in, combining the prior art to achieve the combination of amended limitations; and

(3) improperly discounting Illumina's evidence of unexpected results.

II. STATEMENT OF THE CASE

In IPR2013-00128, IBS challenged Claims 1-8 of Illumina's '026 patent as being either anticipated by or obvious in view of the prior art. JA27-29; JA197. The Board instituted review of all claims on July 29, 2013. JA27-29; JA332-33; JA349.

On February 19, 2014, Illumina filed a Substitute Motion to Amend seeking to cancel Claims 1-8 and replace them with substitute Claims 9-12. JA27-30; JA496-518; 35 U.S.C. § 316(d); 37 C.F.R. § 42.121. Following oral hearing, the Board issued a Final Written Decision cancelling Claims 1-8, but denying Illumina's request to enter substitute Claims 9-12. JA26-57. Illumina appeals the Board's refusal to enter substitute Claims 9-12.

III. STATEMENT OF THE FACTS

A. Technological Background

This appeal relates to innovative, non-natural nucleotide triphosphate molecules used in deoxyribonucleic acid ("DNA") sequence determination methods known as sequencing by synthesis ("SBS"). As relevant to the amended claims, Illumina's innovative nucleotides contain a unique combination of: (1) a disulfide linkage attaching a label to the base; (2) a protecting group attached to the 3'-oxygen atom of the sugar moiety ("3'-protecting group"); and (3) the disulfide linkage and the 3'-protecting group are cleavable under identical conditions. JA501-03.

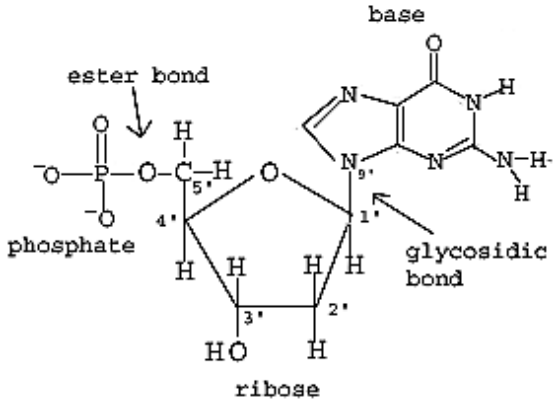
1. Sequencing by synthesis

The '026 patent is directed to non-natural, labeled nucleotides used in SBS. JA37; JA66-67 at 1:12-14, 2:65-3:11. SBS is a term that includes methods of DNA sequencing in which labeled nucleotides are incorporated one-by-one by an enzyme to synthesize a DNA strand. JA70 at 9:15-24; JA1490 at

l.34-JA1491 at l.14. As each nucleotide is sequentially added to the growing DNA strand, the nucleotide is identified according to its label, allowing the sequence of the DNA to be determined. JA70 at 9:15-24; JA1490 at l.34-JA1491 at l.14.

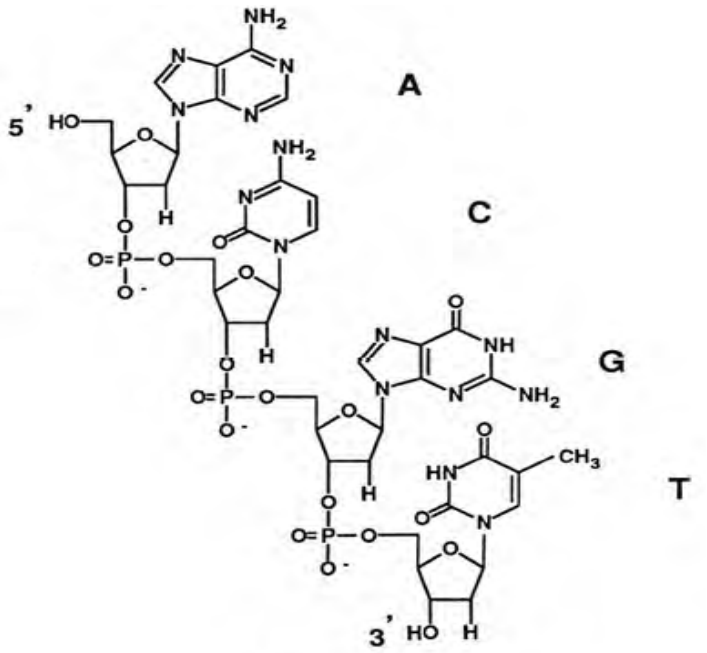
DNA is a linear strand of linked nucleotides whose sequence determines traits about an organism. Determining the sequence of the DNA opens important avenues to diagnose, prevent, and treat numerous diseases and conditions. JA850; JA1485 at ll.18-23.

A nucleotide consists of a nitrogen-containing base, a ribose or deoxyribose sugar moiety, and one or more phosphate groups. JA67 at 4:59-61. In a naturally occurring nucleotide from DNA, the base can be one of four types: adenine ("A"), cytosine ("C"), guanine ("G"), or thymine ("T"). JA67 at 4:63-66; JA1588-89 ¶30. The sugar portion of a nucleotide contains five carbon atoms, which are conventionally numbered 1'-5', as shown below in connection with a G nucleotide:



JA1587-88 ¶28. In their isolated state, nucleotides contain a hydroxyl group ("-OH") at the 3'-position of the sugar, referred to as a "3'-OH." *Id.*

As illustrated below, each DNA strand consists of nucleotides in which the phosphate group of one nucleotide binds with the 3'-OH of the previous nucleotide:



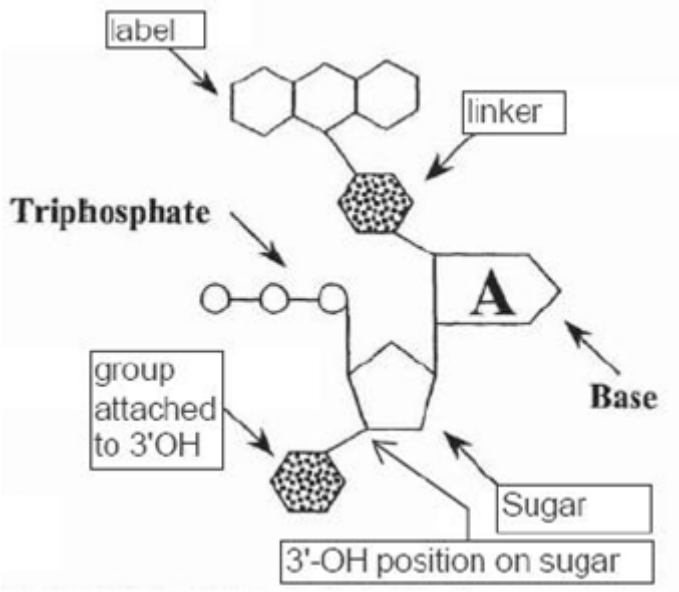
JA5113. DNA within a cell is double-stranded, and the two separate strands bind to one another through hydrogen bonds between the bases of complementary nucleotides in each strand. In this complementary base pairing, A nucleotides in one strand bind to T nucleotides in the other strand, and C nucleotides in one strand bind to G nucleotides in the other strand.

SBS is a process used to determine the nucleotide sequence in a single strand of DNA complementary to a sample single strand of DNA (referred to as

the "template" DNA strand). JA70 at 9:15-24; JA1490 at ll.34-36. This sequence determination occurs by synthesizing the strand of DNA complementary to the template DNA strand, one labeled nucleotide at a time, and identifying each nucleotide that is added to the complementary DNA strand. JA70 at 9:15-34; JA1490 at l.36-JA1491 at l.14. In some SBS methods, the non-natural nucleotides used to synthesize the complementary DNA strand contain a label attached, directly or indirectly (via a linker), to the nucleotide. *See, e.g.*, JA60-62 at Figs. 1-3. These labels are used to identify which nucleotide was added into the complementary DNA strand. JA66 at 2:65-JA67 at 3:11; JA1490 at l.36-JA1491 at l.14.

To facilitate clear identification of each added nucleotide, the addition step is limited to a single nucleotide. This is accomplished by adding a "protecting group" to the nucleotide, which prevents polymerase from incorporating more than one nucleotide. JA69 at 8:11-14. After one identifies the nucleotide added to the complementary DNA strand, the protecting group is removed (also referred to as "deblocked") to regenerate or expose a 3'-OH moiety that allows incorporation of the next nucleotide. JA67 at 3:6-9; JA69 at 8:31-33. Through this sequential process (adding one nucleotide, identifying the added nucleotide, and then deblocking the nucleotide to allow the next nucleotide to be added), the sequence of the template DNA strand can be

determined in a stepwise fashion. JA66 at 2:65-JA67 at 3:11. A generic illustration of a nucleotide having a protecting group at the 3'-position of the sugar and a label attached to the base via a linker is shown below:



JA32.

Ideally, this process of incorporation, identification, and deblocking is repeated for each and every nucleotide in the template DNA strand. Due to the complexity of DNA sequencing methods, an SBS process should be able to determine the sequence of at least 20 consecutive nucleotides in the template DNA strand to be effective. JA4983 at ll.9-12; JA2866 at l.21-JA2867 at l.25; JA2899 at l.14-JA2900 at l.6. The prior art taught SBS methods that achieved much greater sequence lengths. For example, WO 91/06678 ("Tsien") describes in detail SBS methods that allow sequencing of "25 to 300, or more" consecutive nucleotides in the template DNA strand. JA1501 at ll.33-36. Since

each subsequent nucleotide identification relies on the fidelity of the preceding incorporation, identification, and deblocking steps, each of these steps must be performed with very high efficiency to achieve 20 cycles and thereby be useful in SBS. JA1504 at l.24-JA1505 at l.3; JA3958 at 2:22-27; JA2896 at ll.3-8, 14-20.

In SBS processes, the systems often contain numerous identical copies of the same template DNA strand so that several complementary DNA strands are sequenced simultaneously. JA1490 at l.34-JA1491 at l.3; JA1563 at 2:66-JA1564 at 3:2; JA4029 at l.30-JA4030 at l.15. This allows for a more accurate signal measurement by detection equipment and resultant determination of the sequence of the template DNA strand, even if errors are introduced during sequencing in any particular complementary DNA strand.

If the protecting group is not removed efficiently during the SBS process, the protecting group will remain intact in some of the complementary DNA strands, while being removed from others. For those complementary DNA strands with intact protecting groups, the protecting group will continue to block the 3'-OH group, preventing a nucleotide from being added in the next incorporation step. For the other complementary DNA strands in which the protecting groups were removed, the next incorporation step will properly add a nucleotide. When this occurs, all of the complementary DNA strands that did

not properly incorporate the next nucleotide are a round behind ("out of phase") with those that did properly incorporate the next nucleotide. Such out of phase complementary DNA strands produce signal measurements for the wrong nucleotide, which hinders the ability of the SBS method to accurately determine the sequence of the template DNA strand. JA3699-701 ¶50. These errors are multiplicative, in that the strand(s) that go out of phase each round rarely catch up to the proper phase.

Accordingly, nearly 100% efficiency in removing the protecting group is required to prevent complementary DNA strands from becoming out of phase and hindering the usefulness of the method. JA1504 at l.24-JA1505 at l.3; JA3958 at 2:22-27; JA2896 at ll.3-8, 14-20. For example, if a protecting group can be cleaved with only 85% efficiency, 85% of the complementary DNA strands will contain the correct sequence of nucleotides and 15% will not. After just 8 cycles, there would be only $(0.85)^8$, or just 27%, of the complementary DNA strands that contain the correct sequence of nucleotides. JA3699-701 ¶50. The remaining 73% of the complementary DNA strands would be out of phase by one or more rounds because they failed to incorporate a nucleotide during a previous iteration. *Id.* With only 27% of the complementary DNA strands providing the correct signal, and the other strands providing inaccurate signals, accurate identification of the next incorporated nucleotide is not possible. *Id.*

While nearly 100% cleavage efficiency in removing the protecting group is required for SBS, the cleavage efficiency for the linkage connecting a label to the base need not be as high. JA3013 ¶27 (90% cleavage efficiency for the 3'-protecting group is ineffective, but "it is not necessary to cleave the linker with such high efficiency."). In contrast to the cleavage efficiency required for the protecting group, as discussed above, if the linkage connecting the label to the base is not removed in any particular cycle, the next nucleotide will still be properly incorporated into the complementary DNA strand. That complementary DNA strand, however, would contain two labels. Thus, the complementary DNA strand will still be in phase with other complementary DNA strands, even if it provides some ambiguous or incorrect signal from having multiple labels.

Moreover, that strand with two linkers/labels will most likely catch up in subsequent rounds. This means that the label that was not removed during the previous cycle is likely to be removed during the following cycle, and the SBS process will proceed without accumulated error. Accordingly, SBS can proceed with good accuracy even if the cleavage efficiency of the linkage connecting a label to the base does not reach the nearly 100% efficiency required for removing the protecting group.

As a consequence of strands with linkers/labels that are not properly cleaved catching up in subsequent rounds, but strands with uncleaved protecting groups not catching up, skilled artisans tested linkers with lower cleavage efficiency, but had much stricter requirements for protecting group cleavage efficiency.

2. Illumina's '026 patent

SBS is currently the leading method for sequencing DNA, and Illumina is the market leader in this field. JA3795 at ll.8-16; JA820 at l.21-JA821 at l.1 (IBS's counsel referring to Illumina as "the foremost research in, you know, sequencing company in the world."). Illumina's specialized nucleotides were developed through years of scientific research and development efforts.[1]

Illumina filed the '026 patent on August 23, 2002, and it issued on June 6, 2006. JA58. The '026 patent discloses and claims Illumina's nucleotides and corresponding kits containing such nucleotides. JA75. As issued, the '026 patent contained eight claims. *Id.* Independent Claim 1 recites:

> 1. A nucleotide or nucleoside molecule, having a base that is linked to a detectable label via a cleavable linker, wherein the molecule has a ribose or deoxyribose sugar moiety comprising a protecting group attached via the 2' or 3' oxygen atom and the cleavable linker and the protecting group are cleavable under identical conditions. *Id.*

[1] The inventors of the '026 patent were employed at Solexa Ltd. when the '026 patent was filed. Illumina acquired Solexa in 2006. JA201 n.1.

Claim 6 specified that the linker was "acid labile, photolabile or contains a disulphide linkage." *Id.*

When Illumina filed the '026 patent, numerous research groups were searching for improved methods of performing SBS. *See, e.g.*, JA58-75; JA848-97; JA977-1041; JA1277-1304; JA1305-55; JA1483-1544; JA1551-77; JA3945-69; JA4025-84; JA4167-75; JA4176-80; JA5040-87. Illumina's innovative SBS technologies emerged from this crowded field to become the industry-leader for efficient and cost-effective DNA sequencing. JA820 at l.21-JA821 at l.1.

This appeal focuses on the patentability of four claims directed to a narrow class of Illumina's specialized nucleotides and kits containing such nucleotides. Illumina submitted these four claims as substitute Claims 9-12 in its Motion to Amend. JA501-03. Claim 9, which replaces Claim 1, recites:

> 9. A nucleotide triphosphate molecule, having a 7-deazapurine base that is linked to a detectable label via a cleavable linker, wherein the cleavable linker is attached to the 7-position of the 7-deazapurine base and **wherein the cleavable linker contains a disulfide linkage**, and wherein the nucleotide triphosphate molecule has a ribose or deoxyribose sugar moiety comprising **a protecting group attached via the 3' oxygen atom**, and **the disulfide linkage of the cleavable linker and the protecting group are cleavable under identical conditions**. JA3684 ¶22 (emphases added); *see also* JA30; JA501.

3. <u>The prior art</u>

The prior art contains numerous non-natural nucleotides, but none with the unique combination of limitations added to Illumina's substitute claims—a disulfide linkage attaching a label to the base and a 3'-protecting group cleavable under identical conditions as the disulfide linkage.

a. <u>The prior art did not teach nucleotides that combine a 3'-protecting group and a disulfide linkage attaching a label to the base</u>

WO 00/53805 ("Stemple") discloses SBS methods, including methods that utilize a broad genus of nucleotides having a 3'-protecting group that is photolabile (*i.e.*, cleaved by certain forms of light), as well as a label that is attached to the nucleotide base via a photolabile linker. JA852-53; JA887 at Fig. 1. Stemple also discloses 3'-protecting groups and cleavable linkers attaching a label to the base, both of which can be removed enzymatically, chemically, or photolytically. JA853. Stemple, however, does not disclose a

label attached to the base via a disulfide linkage or a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions. JA508.

Similarly, WO 91/06678 ("Tsien") discloses SBS methods, including methods that use a broad genus of nucleotides having a 3'-protecting group and a label attached to the base via a cleavable linkage. JA1490 at l.28-JA1492 at l.7; JA1504 at l.25-JA1505 at l.34. Tsien also describes removing the detectable label and the protecting group "simultaneously." JA1512 at ll.5-8; JA37. Tsien, however, does not disclose the use of a disulfide linkage to attach the label to the base or a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions. JA508; JA40.

U.S. Patent No. 6,664,079 ("Ju") and WO 02/29003 ("Ju PCT") describe SBS methods and nucleotides having a 3'-protecting group and a label. JA1346 at 21:2-16; JA1398 at ll.1-19. Like Tsien and Stemple, Ju does not disclose a label that is connected to the base via a disulfide linkage or a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions. JA508; JA40.

WO 96/27025 ("Rabani") discusses, *inter alia*, various sequencing methods, including SBS methods. JA4025 at Abstract; JA510. Rabani discloses nucleotides having various linkages, including ester linkages, amide linkages, disulfide linkages, photolabile linkages, and thermolabile linkages.

JA4058 at l.9-JA4059 at l.32. Rabani also discloses that 3'-protecting groups can be used in SBS. JA4029 at ll.5-9. Rabani, however, does not disclose or suggest any nucleotides that combine both a 3'-protecting group and a disulfide linkage attaching a label to the base. JA511-12. Moreover, Rabani, despite disclosing 3'-protecting groups and disulfide linkages separately, provides no indication or expectation that the disulfide linkage can be removed under conditions in which a 3'-protecting group usable in SBS is also removable.

U.S. Patent No. 7,785,790 ("Church") and WO 00/53812 ("Church PCT") disclose an SBS method known as Fluorescent In Situ Sequencing Extension Quantification ("FISSEQ"). JA5073 at 44:23-35; JA2635 at ll.1-9. The FISSEQ method does not use 3'-protected nucleotides. JA4853 at l.21-JA4854 at l.9. Church discloses a nucleotide in which a label is attached to the base via a disulfide linkage, but Church's nucleotide does not contain a 3'-protecting group. JA5046 at Fig. 5; JA2678 at Fig. 5.

WO 99/49082 ("Short"), like Church, discloses a nucleotide that contains a disulfide linkage. JA4143; JA4145; JA4151. Short, however, does not disclose a nucleotide that contains both a disulfide linkage and a 3'-protecting group. JA510. Moreover, Short provides no indication or expectation that the disulfide linkage can be removed under conditions in which a 3'-protecting group is also removable.

b. The prior art recognized the importance of deblocking the 3'-protecting group in SBS

The prior art was consistent in recognizing the importance of deblocking the 3'-protecting group in SBS. For example, Tsien discloses an SBS method for sequencing "25 to 300, or more" nucleotides. JA1501 at ll.33-36. In describing this method, Tsien states that "[t]he coupling reaction generally employs 3' hydroxyl-blocked dNTPs to prevent inadvertent extra additions." JA1504 at ll.25-27. Tsien then specifies that "[t]he criteria for the successful use of 3'-blocking groups include . . . the availability of mild conditions for rapid and quantitative deblocking." *Id.* at ll.28-34; JA4856 at ll.2-16 ("'Quantitative' is a term chemists usually use for full, complete, a hundred percent."). Similarly, Ju states that a "fundamental requirement" for SBS methods is the 3'-protecting group and the cleavable linker must be cleavable with high yield. JA1346 at 21:6-13. Additional art, such as U.S. Patent No. 7,078,499 ("Odedra") was consistent with the teachings of Tsien and Ju. *See, e.g.*, JA3958 at 2:22-27 ("For example, if a combined error of approximately 3% in incorporation and cleavage were to accumulate, the result would be that sequence could only be obtained from 5 bases or fewer of the template DNA before the decreased signal to noise ratio made further sequencing impractical."). Sequencing just five bases would be unacceptable for SBS. JA3701 ¶50.

Rabani, while not stating what efficiency is required, teaches that 3'-protecting group cleavage of less than 90% efficiency is "unacceptably low" for SBS, stating:

> The results published by these authors suggests that the rate of chemical removal of 3'-hydroxy protecting groups (less than 90% removal after 10 minutes of treatment with 0.1M NaOH) will be unacceptably low for such an inherently serial sequencing scheme. JA4029 at ll.5-8; *see* JA3699-701 ¶50 (explaining that SBS is a serial sequencing scheme).

The requirements for high 3'-protecting group cleavage efficiency were recognized as a "major challenge" and a "formidable obstacle" for SBS. JA4176-77; JA4167.

c. <u>The prior art taught that disulfide linkage cleavage conditions yielded inefficient and variable results</u>

The prior art that disclosed disulfide linkages in the context of DNA sequencing did not directly address the cleavage efficiency using conditions that cleaved disulfide linkages. Prior art outside of the SBS context, however, showed that disulfide linkage cleavage conditions provided inefficient and variable yields. While these yields might have been acceptable for cleavage of a linkage attaching the label to the base, they would not have been acceptable for cleavage of a 3'-protecting group.

For example, Rabani cites to S.W. Ruby, et al., *Affinity Chromatography with Biotinylated RNAs*, Methods in Enzymology, 181:97-121 (1990) ("Ruby")

in discussing disulfide linkages. JA4058 at ll.29-33; JA4075 (*citing* JA4085-109). Ruby does not disclose SBS methods. JA50 (determining Ruby's disclosure of a disulfide linkage was "not for sequencing purposes"). Ruby describes the use of ribonucleic acid ("RNA")[2] that is biotinylated "for analyzing small nuclear ribonucleoproteins (snRNPs) that bind to precursor messenger RNA (pre-mRNA) while it is being spliced in a yeast *in vitro* system." JA4086. In this context, Ruby discloses an RNA that contains a disulfide linkage. JA4088 (Fig. 2B).

Ruby attempted to optimize the cleavage efficiency of this disulfide linkage using different pH values and various concentrations of the cleavage reagent dithiothreitol ("DTT"). JA4105-06. Ruby's most optimized results provided a maximum disulfide cleavage efficiency of approximately 86%:

[2] RNA has several structural differences from DNA: RNA contains a 2'-OH group, while DNA does not; RNA uses uridine as the complementary nucleotide to adenosine, while DNA uses thymidine as the complementary nucleotide to adenosine.

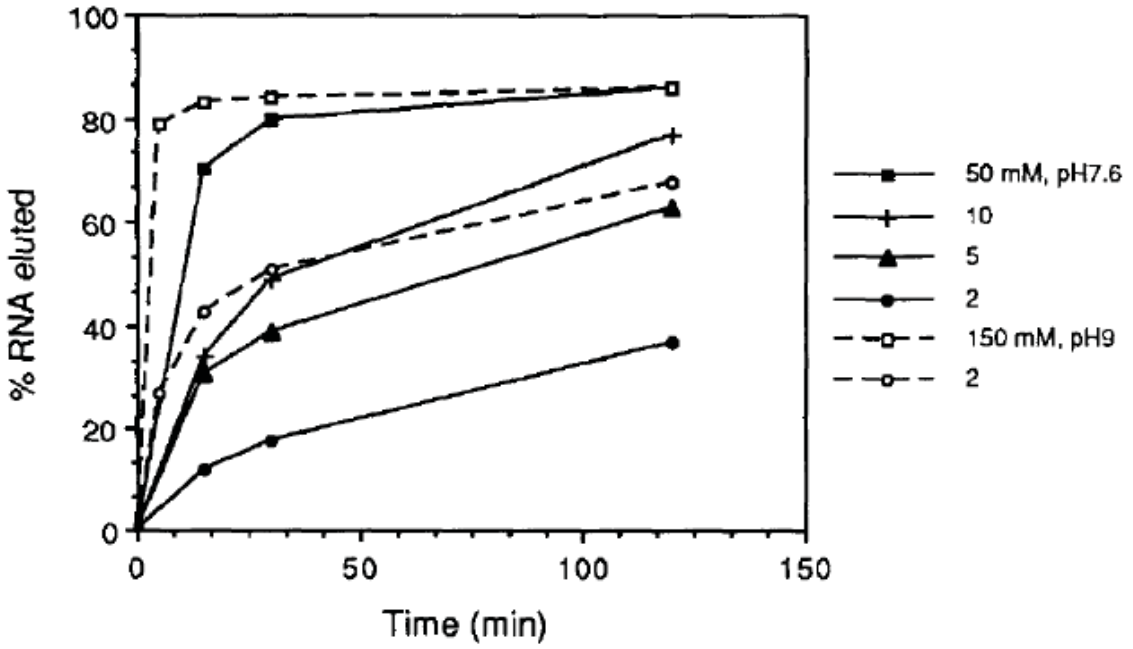


FIG. 4. Eluting biotinylated anchor RNA. Biotinylated anchor RNA 3′-21 was bound to biotin-acryl with succinylavidin as described in Methods. The amounts of RNA eluted with various concentrations of DTT at pH 7.6 (solid lines) or with 2 or 150 m*M* DTT at pH 9 (dashed lines) were measured as a function of time of incubation in the elution buffers.

JA4105; *see also* JA3702-03 ¶54; JA3014 ¶31; JA45.

Numerous publications by Herman *et al.* also discuss the use of a disulfide linkage in contexts other than SBS. JA50 (determining Herman's disclosure of a disulfide linkage was "not for sequencing purposes"). For example, U.S. Patent No. 4,772,691 ("Herman") describes the use of chemically cleavable nucleotides "as hybridization probes and in isolation of target macromolecules from physiological mixtures." JA4112 at 1:7-9. Herman discloses a "Bio-12-SS-dUTP" nucleotide that contains a disulfide linkage, but which does not contain a 3'-protecting group. JA4114. Herman discloses that, after repeated rounds of adding cleavage agent to the disulfide

linkage, the disulfide linkage is cleaved at 87% efficiency. JA4117 at 11:12-16 ("[F]ive consecutive washes of the column containing 1 bound ^{32}P-labeled Bio-SS-DNA with buffer containing 50 mM dithiothreitol resulted in the recovery of a total of 87% of the DNA from the affinity column.").

In 1989, Herman's group reported cleavage of "virtually all" of the disulfide linkage from "Bio-19-SS-dUTP," but failed to provide any supporting data. JA2561. In later experiments, Herman's group stated that "approximately one-half" of the disulfide linkage cleaved and provided data supporting this conclusion. JA5120; JA5124 (bar graph). Other researchers also experienced variable results using disulfide linkages with Bio-19-SS-dUTP nucleotides. In Rigas, et al., *Rapid Plasmid Library Screening Using Reca-Coated Biotinylated Probes,* PNAS USA, 83:9591-95 (1986) ("Rigas"), the authors concluded that "reduction of the disulfide bond of Bio-19-SS-dUTP gave variable results" and "was not pursued rigorously." JA5131. The patent literature reported this same variability. JA5139 at 6:61-64.

B. The *Inter Partes* Review Proceedings

1. IBS's petition and the Board's institution decision

In the related district court litigation, *Trustees of Columbia Univ. v. Illumina, Inc.*, Case No. 1:12-cv-00376, (D. Del.), Illumina has alleged that IBS infringes the '026 patent. *See, e.g.,* JA3359. On January 29, 2013, IBS

petitioned for *inter partes* review of the '026 patent. JA118; JA181. On February 7, 2013, IBS submitted a revised petition, correcting certain defects identified by the Board. JA190-91; JA197; JA260.

IBS's revised petition challenged all claims of the '026 patent as anticipated or obvious in view of Stemple, Ju, Tsien, and several other prior art references. JA205-08. Illumina filed a preliminary response on May 1, 2013. JA285; JA329.

On July 29, 2013, the Board issued its decision instituting *inter partes* review. JA332-50. Specifically, the Board instituted review of Claims 1-6 as anticipated by Tsien and Ju, Claim 3 as obvious in view of Tsien and Prober et al., *A System for Rapid DNA Sequencing with Fluorescent Chain-Terminating Dideoxynucleotides*, Science 238:336 (1987) ("Prober"), and Claims 7-8 as obvious in view of Ju or Tsien combined with another reference, but declined to institute review on the other bases raised by IBS. JA333-34; JA340; JA342-45; JA347-49.

In analyzing the prior art, the Board focused on Tsien and Ju. JA337-48. The Board concluded that Tsien "discloses a sequencing-by-synthesis method" and nucleotides having a 3'-protecting group and a label attached to the base via a cleavable linker. JA337-38. The Board concluded that "Tsien also teaches that 'a fluorescent tag attached to the base moiety . . . may be chemically

cleaved (either separately or **simultaneously** with the deblocking step)'," satisfying the requirement of Claim 1 that the protecting group and the linker are cleavable under identical conditions. JA339.

Similarly, the Board concluded that Ju "discloses nucleic acid sequencing by synthesis methods, which utilize nucleotide analogs that include a fluorescent label attached to the nucleotide analogs through a cleavable linker and a cleavable chemical group to cap the –OH group at the 3' position (3'-OH) of the deoxyribose." JA343. The Board also concluded that Ju "describes the same means to cleave the label and the chemical moieties from the nucleotide," satisfying the cleavable under identical conditions limitation. JA344.

2. Illumina's Motion to Amend

In response to the Board's institution decision, Illumina filed a Motion to Amend, supported by the expert declaration of Dr. Romesberg and a declaration of Eric Vermaas, describing testing performed by Illumina.[3] JA496; JA500-01; JA3679-715; JA4572-85. Illumina's Motion sought to cancel Claims 1-8 and add proposed substitute Claims 9-12. JA500-03. Illumina explained in detail that substitute Claims 9-12 were narrower in scope than Claims 1-8 and were

[3] Illumina filed its Motion to Amend and supporting declarations on October 24, 2013. JA484. With the Board's permission, JA481, Illumina filed a substitute Motion to Amend and supporting declarations to correct non-substantive errors on February 19, 2014, JA80.

fully supported by the patent's written description. JA501-05; *see* JA3685-93 ¶¶26-33; 37 C.F.R. § 42.121.

Illumina also explained that the Board's grounds for instituting trial were moot in view of the substitute claims because "no combination of [the references on which the Board instituted review] would afford a nucleotide triphosphate molecule that has a disulfide linkage, much less the claimed nucleotide triphosphate molecule comprising a disulfide linkage and a 3'-OH protecting group that are cleavable under identical conditions." JA507. Illumina also argued that the other prior art cited by IBS and the Board does "not disclose or suggest a cleavable linker that 'contains a disulfide linkage' or conditions that would cleave both a disulfide linkage and a 3'-OH protecting group under identical conditions." JA508; *see also* JA509-10.

Illumina then independently identified Rabani, Ruby, Herman, and Short, which discuss disulfides. JA510. Illumina explained that none of these references, alone or in combination with the other prior art identified by IBS or the Board, disclose or suggest choosing a **3'-protecting group that is cleavable under identical conditions with a disulfide linkage**. *Id.* Moreover, Illumina extensively outlined why the prior art provided "no indication that a cleavable 3'-OH protecting group *cleavable under identical conditions* to a disulfide linkage (as claimed) would cleave with [the efficiency required for

SBS]." JA511; *see* JA510-13; JA3697-705 ¶¶45-60. Finally, Illumina presented evidence showing that the nucleotides of the substitute claims demonstrated an unexpectedly high and surprisingly rapid cleavage rate for the disulfide linkage under identical conditions to 3'-protecting group cleavage compared to what would have been expected in view of the prior art. JA513-16; JA4573-84 ¶¶6-19; JA3705-14 ¶¶61-74.

On January 24, 2014, IBS opposed Illumina's Motion to Amend. JA449. Illumina replied on February 24, 2014. JA547. The Board conducted an oral hearing on April 23, 2014. JA784-85.

3. The Board's Final Written Decision

On July 25, 2014, the Board issued its Final Written Decision cancelling Claims 1-8 and denying Illumina's Motion to Amend with respect to substitute Claims 9-12. JA26-27. In addressing Illumina's Motion to Amend, the Board focused on the disulfide linkage limitation, stating:

> None of the original claims, however, comprised the limitation that the cleavable linker "contains a disulfide linkage." The obviousness of using a disulfide linkage is the main issue to be decided in whether to grant the Motion to Amend. JA30.

Notably, while addressing the disulfide linkage limitation that Illumina added to the claims, the Board failed to address the other limitations that Illumina added in that amendment. *See* JA501 (proposed Claim 9 showing additions in underline and deletions in strikethrough). The Board did not address the

combination of amended limitations in Illumina's substitute claims or provide any motivation for combining the elements of the prior art to achieve that combination. Specifically, the Board did not provide any motivation for combining the prior art to arrive at the claimed nucleotide having a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions. Indeed, the Board did not identify any specific 3'-protecting group that is cleavable under identical conditions as a disulfide linkage attaching a label to the base, let alone one that met the efficiency requirements for SBS.

In addressing the disulfide linkage limitation, the Board concluded that "Rabani and Church both describe attaching a detectable label to a nucleotide base via a disulfide linkage, where the nucleotide is used in nucleic acid sequencing." JA37-38. The Board found that Rabani "would have given a skilled worker reason to have used a cleavable linker with a disulfide bond to ensure that the labeling moieties on the nucleotides will not interfere with the action of a polymerase enzyme during the synthesis reaction," and that Church further established the conventionality of a disulfide linker to attach a label to the base of a nucleotide. JA41.

Illumina argued that one skilled in the art would not have been motivated to combine a 3'-protecting group and a disulfide linkage attaching a label to the

base that are cleavable under identical conditions. In such a combination, one skilled in the art would not have expected the 3'-protecting group to cleave with sufficient efficiency for SBS. JA42-44. The Board appears to have misapprehended Illumina's arguments. First, the Board incorrectly stated, "Illumina contends sequencing by synthesis processes require that **the disulfide linker** joined to the detectable label **be cleaved with 90% efficiency**, because of the iterative nature of the process." JA42 (emphases added). That was not Illumina's argument. Illumina argued that the **3'-protecting group** must be cleaved with greater than 90% efficiency, and that one skilled in the art would not have expected to achieve that efficiency when combining a 3'-protecting group and a disulfide linkage that are cleavable under identical conditions. Second, the Board incorrectly identified the location of the disulfide bond, repeatedly referring to the "disulfide linkage at the 3'-OH group." JA43. None of the parties (or the cited art) referenced a nucleotide having a disulfide linkage as part of the 3'-protecting group. The disulfide linkage in the substitute claims attaches the label to the base. The Board also concluded that "Illumina has not met its burden to show that cleavage of the disulfide bond, attaching the detectable label to the base, with less than 90% efficiency would be unacceptable for sequencing." JA44. Illumina made no such argument and bore no such burden.

The Board recognized the inefficient and variable cleavage for disulfide linkages in the prior art. JA45-49. Nevertheless, the Board concluded that "even if 90% efficiency were necessary for a reasonable expectation of success, the ordinary artisan would have expected that such cleavage efficiency of the disulfide bond could be achieved." JA50. The Board premised this conclusion exclusively on the unsupported testimony of IBS's expert and the Board's unsupported interpretation of the prior art. JA44-50. Neither supports this conclusion.

The Board also stated that "Illumina has not met its burden to show that identical conditions could not be selected in which the disulfide linkage of the cleavable linker is cleavable with less than 90% efficiency and the protecting group is cleavable with greater than 90% efficiency as required by the proposed substitute claims." JA50. This argument, however, was not raised by the parties, and the evidence of record does not suggest or teach that such conditions could be identified with a reasonable expectation of success. Even with the benefit of hindsight, the evidence of record fails to establish if such conditions even exist.

Finally, the Board addressed Illumina's evidence of unexpected results. In doing so, the Board apparently faulted Illumina's comparison, noting that "Illumina does not state what references are the closest prior art to the claims."

JA52. The Board then stated that Illumina's testing was insufficient because the results may be a latent property of the disulfide linkage. JA54.

Illumina timely filed its notice of appeal on September 24, 2014. *See* 35 U.S.C. § 142; 37 C.F.R. § 90.3(a).

IV. SUMMARY OF THE ARGUMENT

The Board erred in analyzing Illumina's proposed substitute Claims 9-12 and in denying entry of these claims in the '026 patent based on the conclusion that they would have been obvious.

In assessing whether Claims 9-12 would have been obvious, the Board focused almost exclusively on a single limitation—the requirement that the cleavable linker connecting the label to the base "contains a **disulfide linkage**." The Board went so far as to state that "[t]he obviousness of using a disulfide linkage is the main issue to be decided in whether to grant the Motion to Amend." JA30; *see also* JA35.

The Board never addressed the combination of amended limitations in Claims 9-12, despite this combination being the focal point of Illumina's arguments and the central point of dispute. Tellingly, the Board never addressed the combination of a disulfide linkage attaching a detectable label to the base and **a 3'-protecting group cleavable under identical conditions to the disulfide linkage**. The Board's Final Written Decision does not even set

forth the combination of references which, in the Board's view, would have rendered Illumina's claims obvious.

The Board's improper focus on the disulfide linkage limitation, rather than the combination of a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions, also infected its analysis of motivation to combine and reasonable expectation of success. While the Board attempted to identify a motivation for, and a reasonable expectation of success in, using a disulfide linkage in nucleotides generally, the Board presented no such analysis for the claimed combination of a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions. The record shows that no such motivation or expectation of success existed at the time of the invention.

The experts for both parties agreed that SBS requires that the 3'-protecting group of the nucleotides be removed with greater than 90% efficiency. The evidence showed that over 97%, and preferably nearly 100%, removal efficiency of the 3'-protecting group is required. The prior art, consistent with the expert testimony, teaches that less than 90% efficiency is insufficient and suggests that even higher efficiency is required. Illumina's expert, Dr. Romesberg, explained that in a nucleotide having a 3'-protecting group and a base-attached disulfide linkage that are cleavable under identical conditions, one skilled in the art would not expect the 3'-protecting group to

cleave with any greater efficiency than the disulfide linkage. Neither IBS nor its expert challenged this testimony; it stood unrebutted.

In the context of SBS and outside of this context, the prior art showed inefficient (less than 90%) and variable cleavage of disulfide linkages. Accordingly, in light of Dr. Romesberg's unrebutted testimony, one skilled in the art would not have expected to achieve greater than 90% (let alone 97-100%) cleavage efficiency of the 3'-protecting group in a nucleotide containing a 3'-protecting group and a disulfide linkage that are cleavable under identical conditions. Thus, there was no motivation for, or expectation of success in, combining the prior art to achieve the subject matter of Illumina's substitute claims.

The Board misapprehended Illumina's arguments regarding 3'-protecting group cleavage efficiency. This is apparent from the Board's repeated reference to "the disulfide linkage at the 3'-OH group" and the "disulfide bond on the protecting group." JA43. Not one reference cited in the proceedings disclosed or suggested a 3'-protecting group containing a disulfide linkage, and there is no evidence that such a 3'-protecting group existed. In the substitute claims, the disulfide linkage is part of the cleavable linker attaching the label to the base, and it is wholly separate from the 3'-protecting group.

The Board also erred in concluding that one skilled in the art could have increased cleavage efficiency with a reasonable expectation of success. The Board applied the wrong standard, requiring Illumina to prove that a skilled artisan would have been unable to increase cleavage efficiency. JA44; JA50. Moreover, the Board effectively determined that because the prior art demonstrated widely variable cleavage efficiency of disulfide linkages, one skilled in the art would have had a reasonable expectation of success in increasing such cleavage efficiencies to greater than 90%, thereby increasing the cleavage efficiency of the 3'-protecting group to the level necessary for SBS. The evidence does not support the Board's conclusion. Rather, the evidence shows that one skilled in the art would not have had a reasonable expectation of success in increasing the disulfide linkage cleavage efficiencies of the prior art.

The Board's Final Written Decision is bereft of any motivation for, or expectation of success in, combining the prior art to achieve a nucleotide having a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions. No such motivation or expectation of success is present in the record. Moreover, the Board's decision failed to identify any specific prior art 3'-protecting group and disulfide linkage that were cleavable under identical conditions.

The Board also erred in discounting Illumina's evidence of unexpected results. Illumina provided testing and expert analysis showing that the claimed nucleotides demonstrate unexpectedly efficient and rapid cleavage of the 3'-protecting group and the disulfide linkage. The Board faulted Illumina for failing to identify the closest prior art to the claims. Illumina, however, systematically analyzed the prior art and compared the cleavage efficiency of the disulfide linkage of its claimed nucleotides to the only prior art of record that contained cleavage efficiency information for disulfide linkages. The Board also faulted Illumina for allegedly not demonstrating that Illumina's unexpected results were not a latent property of the disulfide linkage. The Board's latent property position is incorrect because Illumina's claimed nucleotides were not known in the prior art.

Given the flaws in the Board's obviousness analysis, the Board erred in denying Illumina's Motion to Amend.

V. ARGUMENT

A. Standard Of Review

This Court reviews the Board's legal conclusions *de novo*, and its factual findings for substantial evidence. *Rambus Inc. v. Rea*, 731 F.3d 1248, 1251 (Fed. Cir. 2013). Substantial evidence review "involves examination of the record as a whole, taking into account evidence that both justifies and detracts

from an agency's decision." *In re Gartside*, 203 F.3d 1305, 1312 (Fed. Cir. 2000). "Substantial evidence is more than a mere scintilla. It means such relevant evidence as a reasonable mind might accept as adequate to support a conclusion." *Id.* (*quoting Consolidated Edison Co. v. NLRB*, 305 U.S. 197, 229-30 (1938)).

Obviousness is a legal question based on underlying factual determinations, including: "1) the scope and content of the prior art, 2) the level of ordinary skill in the art, 3) the differences between the claimed invention and the prior art, and 4) evidence of secondary factors, also known as objective indicia of non-obviousness." *Eisai Co. Ltd. v. Dr. Reddy's Labs., Ltd.*, 533 F.3d 1353, 1356 (Fed. Cir. 2008).

B. The Board Erred In Determining That Substitute Claims 9-12 Would Have Been Obvious

The Board's conclusion that substitute Claims 9-12 would have been obvious was premised on several errors. First, the Board improperly focused on a single added claim limitation—the disulfide linkage limitation—rather than the combination of amended limitations, specifically, a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions. Second, the Board failed to identify a motivation for, or expectation of success in, combining the elements of the prior art to achieve

Illumina's claimed combination. Finally, the Board impermissibly discounted Illumina's evidence of unexpected results.

1. The Board improperly focused on the added disulfide linkage limitation rather than combination of amended claim limitations

Virtually all patented inventions comprise a combination of several claim limitations. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418-19 (2007) ("claimed discoveries almost of necessity will be combinations"); *Envtl. Designs, Ltd. v. Union Oil Co.*, 713 F.2d 693, 698 (Fed. Cir. 1983). A claimed combination is not obvious if there was no "reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does." *KSR*, 550 U.S. at 418. A claim cannot be held obvious without considering the combination of features in the manner claimed. *Id.* ("a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art."); *Ruiz v. A.B. Chance Co.*, 357 F.3d 1270, 1275 (Fed. Cir. 2004).

In analyzing Claims 9-12, the Board failed to heed these principles and focused on the disulfide linkage limitation, instead of analyzing the combination of amended limitations, particularly a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions. For example, the Board stated:

> None of the original claims, however, comprised the limitation that the cleavable linker "contains a disulfide linkage." **The obviousness of using a disulfide linkage is the main issue to be decided in whether to grant the Motion to Amend**. JA30 (emphasis added).[4]

Similarly, the Board stated, "The **main limitation at issue** in Illumina's Motion to Amend is the recitation in all the proposed substitute claims that a 7-deazapurine base is linked to a detectable label through a 'cleavable linker [which] contains a **disulfide linkage**.'" JA35 (emphases added; brackets in original). Thus, the Board erred by focusing exclusively on "the obviousness of using a disulfide linkage," rather than the combination of a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions. JA30.

The Board never addressed the combination of a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions. The Board erred by analyzing the disulfide linkage limitation in isolation, rather than analyzing all of the amendments made to the claim, as mandated by this Court's precedent. *See Schenck v. Nortron Corp.*, 713 F.2d 782, 785 (Fed. Cir. 1983).

When all of the amended limitations are properly considered in combination, Illumina's substitute Claims 9-12 would not have been obvious.

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[4] The Board's statement that none of the original claims contained the "disulfide linkage" limitation is incorrect. JA30. Claim 6 expressly recited that "the linker is acid labile, photolabile or contains a **disulphide linkage**." JA75 (emphasis added).

2. The Board failed to provide a motivation for, or a reasonable expectation of success in, combining the prior art to achieve Illumina's claimed invention

"[A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, 550 U.S. at 418. Rather, obviousness requires proof "that a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so." *Procter & Gamble Co. v. Teva Pharms. USA, Inc.*, 566 F.3d 989, 994 (Fed. Cir. 2009) (*quoting Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1361 (Fed. Cir. 2007)).

Illumina's substitute claims are directed to nucleotides, and kits containing nucleotides, that uniquely combine a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions. JA501-03; JA30. While it may be possible to independently identify each of these limitations by culling through the prior art, there was no motivation or reasoned approach that would have led a skilled artisan to combine these limitations in a nucleotide triphosphate molecule. In analyzing Illumina's substitute claims, the Board was erroneously guided by hindsight to identify claim limitations in the prior art, rather than to assess the patentability of the combination of amended limitations. *See Ruiz*, 357 F.3d at 1275 ("This

form of hindsight reasoning, using the invention as a roadmap to find its prior art components, would discount the value of combining various existing features or principles in a new way to achieve a new result—often the very definition of invention."); *KSR*, 550 U.S. at 421.

The Board focused on the disulfide linkage limitation and concluded that Rabani discloses disulfide linkages and "would have given a skilled worker reason to have used a cleavable linker with a disulfide bond to ensure that the labeling moieties on the nucleotides will not interfere with the action of a polymerase enzyme during the synthesis reaction." JA41. The Board also relied on Church as "provid[ing] another example of the use of a disulfide linker to attach a label to base of a nucleotide, further establishing its conventionality at the time of the invention." *Id.*

The Board's analysis with respect to motivation to modify is insufficient. The Board failed to address the other amended limitations of the claims and also failed to provide motivation for using the combination of a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions. As Illumina properly demonstrated, there was no such motivation at the time of the invention.

The Board properly recognized that "[t]he claimed nucleotides are used in nucleic acid SBS." JA37. Based on the evidence of record, the only

potential reason for combining or modifying the prior art references is to create nucleotides for use in SBS. JA508; JA455-56; JA459-60; JA228-30; JA239-41; JA249-50; JA258. Indeed, the only motivation for modifying or combining the prior art proffered by IBS was "to **improve the sequencing by synthesis methods**" of the prior art. JA1596-97 ¶51 (emphasis added); JA1605-06 ¶75; JA1610 ¶89; JA1616 ¶106.

It was recognized in the art that each cycle of an SBS method must exhibit high efficiency. *See, e.g.*, JA3958 at 2:22-27. In particular, the step of removing the 3'-protecting group must proceed at greater than 90% efficiency, closer to 97-100% efficiency. JA510-11; JA4029 at ll.5-8 (less than 90% cleavage efficiency for the 3'-protecting group "will be unacceptably low for such an inherently serial sequencing scheme."); JA3699-701 ¶50; JA3013 ¶27; JA462-63; *see also* JA1504 at ll.28-34; JA1346 at 21:3-13. A skilled artisan would not have had a reasonable expectation that combining a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions would result in a nucleotide in which the 3'-protecting group cleaves with greater than 90% efficiency, let alone the 97-100% required for SBS. JA510-13; JA3702-05 ¶¶52-55, 58, 60. Therefore, there would have been no motivation to combine the prior art references to arrive at Illumina's substitute claims. *See DePuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, 567 F.3d 1314, 1326 (Fed. Cir.

2009) (explaining that a claim is nonobvious when "the prior art indicated that the invention would not have worked for its intended purpose or otherwise taught away from the invention.") (*citing United States v. Adams*, 383 U.S. 39, 52 (1966)).

The Board erred in determining that there was a motivation to combine the prior art. First, the Board misapprehended Illumina's argument regarding the 3'-protecting group cleavage efficiency required for SBS. Second, the Board erred by failing to provide any motivation to use a disulfide linkage in combination with a 3'-protecting group that are cleavable under identical conditions, especially where the evidence would not have supported such a conclusion. Finally, the Board erred in finding an expectation of success in manipulating disulfide linkage cleavage conditions to achieve sufficient cleavage efficiency of the 3'-protecting group for SBS.

a. <u>The uncontroverted evidence showed that SBS requires efficient removal of the 3' protecting group</u>

The Board did not expressly determine the cleavage efficiency of the 3'-protecting group necessary for SBS. The Board, however, conducted its analysis as if 90% cleavage efficiency was sufficient based on Rabani, noting that:

> Rabani disclosed published results "suggest[ing] that the rate of chemical removal of 3'-hydroxy protecting groups (less than 90% removal after 10 minutes of treatment with 0.1M NaOH) will be unacceptably low for such an inherently serial sequencing scheme." JA42 (brackets in original) (*quoting* JA4029 at ll.6-8).

But Rabani does not discuss the minimum 3'-protecting group cleavage efficiency required for SBS. Rather, Rabani merely observed that particular removal conditions produced less than 90% cleavage efficiency, which is "unacceptably low."

Contrary to the Board's analysis, the undisputed evidence demonstrates that 90% cleavage efficiency of the 3'-protecting group is insufficient for SBS, and that SBS requires 3'-protecting group cleavage efficiency approaching 100%. *See* Section III.A.3.b *supra*. The Board erred by not evaluating the art (and Illumina's arguments) using the 3'-protecting group cleavage efficiency required for SBS, namely 97-100%.

IBS's expert, Dr. Branchaud, acknowledged that 90% cleavage efficiency is insufficient, testifying that "**I agree that a person of skill in the art would want greater than 90% efficiency for cleaving the 3'-OH protecting group** . . . ." JA3013 ¶27 (emphasis added); *see also* JA462-63. Illumina's expert, Dr. Romesberg, testified that "less than 90% efficiency is unacceptably low for use in iterative DNA sequencing, such as SBS." JA3701 ¶50. Thus, the experts

agreed that less than 90% cleavage efficiency of the 3'-protecting group is insufficient for SBS.

In addressing the cleavage efficiency required for the 3'-protecting group in SBS, the Board noted that Dr. Romesberg "conceded that he did not choose a 90% efficiency requirement because of Rabani, but rather because 'it was a round number slightly above the values reported by Ruby and Herman.'" JA43-44. But Dr. Romesberg's discussion of 90% cleavage efficiency, on which the Board relied, was not a discussion of the cleavage efficiency necessary for SBS, but rather an explanation of why 90% cleavage efficiency was insufficient. This is apparent from Dr. Romesberg's testimony, where he explained that the actual cleavage efficiency of the 3'-protecting group required for SBS is much higher than 90%—it is close to 100%:

> I never suggested that greater than 90 percent was sufficient. It's certainly necessary. But necessary is not necessarily sufficient. And 91 percent is greater than 90, but that would not be sufficient. Ninety-five percent would not be sufficient. Sufficiency is really something approaching 100. JA2896 at ll.14-20.

Dr. Romesberg's testimony that the 3'-protecting group cleavage efficiency required for SBS is far above 90%, and closer to approaching 100%, is uniformly supported by the prior art. For example, Tsien recognizes that "[t]he criteria for the successful use of 3'-blocking groups include . . . the availability of mild conditions for rapid and quantitative deblocking . . . ."

JA1504 at l.24-JA1505 at l.3. Quantitative deblocking is nearly 100% removal of the 3'-protecting group. JA4856 at ll.2-16 ("'Quantitative' is a term chemists usually use for full, complete, a hundred percent."). Tsien goes on to explain that:

> After successfully incorporating a 3'-blocked nucleotide into the DNA chain, the sequencing scheme requires the blocking group to be removed to yield a viable 3'-OH site for continued chain synthesis. The deblocking method should:
> (a) proceed rapidly,
> (b) yield a viable 3'-OH function in high yield . . . .
> JA1507 at ll.28-35.

Ju similarly states that a "fundamental requirement" for SBS is that the 3'-protecting group must be cleavable with "high yield." JA1346 at 21:6-13. Odedra teaches that the cleavage efficiency should be at least 97%. JA3958 at 2:22-27. Importantly, neither IBS nor its expert disputed that SBS requires nearly 100% cleavage efficiency for the 3'-protecting group. *See* JA4989 at l.24-JA4992 at l.11 (IBS's expert refusing to testify regarding the 3'-protecting group cleavage efficiency a skilled artisan would require for SBS).

The evidence shows that for SBS the 3'-protecting group must be removed with greater than 90% cleavage efficiency, and likely close to 100% cleavage efficiency. No evidence of record supports a lower cleavage efficiency requirement for the 3'-protecting group. The Board erred in failing to address the evidence in view of the unchallenged cleavage efficiency

required for SBS, instead applying a much lower requirement to find obviousness.

b. The Board misapprehended Illumina's argument regarding cleavage efficiency

The Board stated that "Illumina contends sequencing by synthesis processes require that **the disulfide linker** joined to the detectable label be cleaved with 90% efficiency." JA42 (emphasis added) (*citing* JA510-11). This was not Illumina's position. Rather, in accord with the undisputed expert testimony and the teachings of the prior art, Illumina asserted that SBS requires that **the 3'-protecting group** be cleaved with greater than 90% efficiency, and likely between 97-100% efficiency. *See* JA2896 at ll.14-20 (SBS requires "something approaching 100" percent efficiency); JA510 ("Rabani teaches that less than 90% cleavage of a 3'-OH protecting group is unacceptably low for DNA sequencing by synthesis ('SBS')."); *see also* JA511 ("Rabani, Ruby, Herman, and Short provide no indication that a cleavable 3'-OH protecting group *cleavable under identical conditions* to a disulfide linkage (as claimed) would cleave with this required efficiency.") (emphasis in original); JA3699-701 ¶50; JA3013 ¶27.

In its Motion to Amend, Illumina argued that when the 3'-protecting group of a nucleotide is cleavable under identical conditions to a disulfide linkage attaching a label to the base, there is nothing in the prior art that would

have led one skilled in the art to expect that the 3'-protecting group would cleave with any greater efficiency than the disulfide linkage. JA512. Illumina's argument was supported by the testimony of Dr. Romesberg, who testified that the prior art provided no indication or expectation that a 3'-protecting group that is cleavable under identical conditions as a disulfide linkage would be cleaved with any greater efficiency than the disulfide linkage. JA3701-05 ¶¶52, 55, 58, 60. Dr. Romesberg's testimony was based on his evaluation of the prior art and his background as an expert in SBS. JA3680-83 ¶¶2-17.

IBS's expert did not challenge Dr. Romesberg's testimony on this key point. Instead, Dr. Branchaud testified:

> I agree that a person of skill in the art would want greater than 90% efficiency for cleaving the 3'-OH protecting group; however, if the cleavable linker is being used to attach a label to the base of the nucleotide, as in the Proposed Substitute Claims, although it might be desirable, it is not necessary to cleave the linker with such high efficiency. JA3013 ¶27.

While recognizing that 90% cleavage efficiency of the 3'-protecting group was insufficient, Dr. Branchaud did not challenge Dr. Romesberg's testimony that a skilled artisan would not have expected the 3'-protecting group to cleave with greater efficiency than the disulfide linkage under identical conditions. *Id.* Thus, Dr. Romesberg's testimony on this point, the only evidence of record, stands unchallenged.

The prior art demonstrates that disulfide linkages cleave with less than 90% efficiency. *See infra* at Section V.B.2.c. Accordingly, Illumina argued that a skilled artisan would not have been motivated to combine a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions. A skilled artisan would not have expected the 3'-protecting group to cleave with any greater efficiency than the disulfide linkage, which is below 90% and well below the nearly 100% 3'-protecting group cleavage efficiency necessary for SBS. JA3701-05 ¶¶52, 55, 58, 60; *see also* JA512.

The Board misapprehended Illumina's argument. This is apparent from the Board's discussion of the issue:

> In other words, Illumina's argument is that since 90% efficiency in cleaving the **disulfide linkage at the 3'-OH group** could not be achieved, there would not have been a reason to use a disulfide linkage to attach the detectable label to the base, because the detectable label must [be] cleaved under identical conditions to the 3'-OH protecting group. Cleavage of the **disulfide linkage at the 3'-OH group** requires 90% efficiency Illumina argues 90% cleavage efficiency must be achieved at the disulfide bond of the detectable label, as well.
>
> Illumina's argument is flawed. Rabani's disclosure is directed to cleavage of the protecting groups, not the detectable label as claimed. Illumina's arguments are based on the logic that if no better than 90% cleavage of the **disulfide bond on the protecting group** can be achieved, the skilled worker would not have used it as a cleavable linker for attaching a detectable label to a nucleotide in DNA sequencing, because the proposed substitute claims require it be cleaved under identical conditions to the 3'-OH group, which requires 90% efficiency.

> The proposed substitute claims do not require the **linkage between the 3'-OH and protecting group to comprise a disulfide bond**. We, therefore, discern no reason to apply Rabani's protecting group cleavage efficiency requirement to the disulfide linkage of the proposed substitute claims. JA43 (bold emphases added).

The Board's misunderstanding of Illumina's position is demonstrated by the Board's repeated reference to a disulfide linkage at the 3'-protecting group. No such linkage was at issue in the parties' arguments or the prior art addressed by the parties. The Board's analysis ignores the uncontroverted expert testimony and provides no basis for denying Illumina's Motion to Amend.

As discussed above, the uncontroverted evidence shows that the 3'-protecting group must be cleaved with much greater than 90% efficiency (close to 100%) for SBS. One skilled in the art would not have been motivated to combine the prior art references for SBS unless there was a reasonable expectation that the combination would provide a 3'-protecting group that cleaves with nearly 100% efficiency. Notably, neither the Board nor IBS identified any 3'-protecting groups cleavable under identical conditions to a disulfide linkage that met this efficiency requirement. Absent such motivation, the Board's conclusion that substitute Claims 9-12 would have been obvious is unsupported. *See DePuy*, 567 F.3d at 1326 ("An inference of nonobviousness is especially strong where the prior art's teachings undermine the very reason

being proffered as to why a person of ordinary skill in the art would have combined the known elements.").

c. <u>One skilled in the art would not have been motivated to use disulfide linkage cleavage conditions because they result in inefficient and variable cleavage</u>

The prior art provides an expectation of low and unpredictably variable cleavage efficiency when using conditions that cleave disulfide linkages. Either low or variable cleavage efficiency would have dissuaded a skilled artisan from combining a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions because such a 3'-protecting group would have been expected to cleave with no greater efficiency than the disulfide linkage—an efficiency insufficient for SBS. *In re ICON Health & Fitness, Inc.*, 496 F.3d 1374, 1382 (Fed. Cir. 2007) ("a reference teaches away from a combination when using it in that combination would produce an inoperative result").

The prior art teaches that disulfide linkages, within and outside of the context of SBS, cleave with less than 90% efficiency, and far less than 97-100% efficiency. For example, in discussing disulfide linkages in the context of SBS, Rabani cites to Ruby. JA45; JA4058 at ll.29-33 (*citing* JA4085-91 (Ruby)); JA3701 ¶51. Ruby discloses an RNA that contains a disulfide linkage for an application unrelated to DNA sequencing. JA50. Ruby tested numerous

reaction conditions to achieve a maximum disulfide cleavage efficiency of approximately 86% after more than 100 minutes. JA4105 (Fig. 4); JA45; JA3702-03 ¶54; JA3014 ¶31.[5]

In the context of SBS, Church discloses cleavage of a disulfide linkage in Figure 6. This figure, however, is of such poor quality that the extent of disulfide cleavage cannot be determined, and the Board correctly gave this figure no weight. JA49; JA2679 (Fig. 6). Therefore, Church would not have provided an expectation that nucleotides with a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions would produce cleavage of the 3'-protecting group with sufficient efficiency for SBS.

Herman, a reference not directed to DNA sequencing, discloses a "Bio-12-SS-dUTP" nucleotide with a disulfide linkage that cleaves with 87%

[5] Rabani also cites The Pierce Catalog ("Pierce") for disulfide linkages. JA40-41; JA4058 at ll.29-33; JA4075. Pierce discloses many cross-linking reagents for *protein* chemistry. JA4932 at l.10-JA4933 at l.14; JA2961-98. The Board did not rely on any particular reagent from Pierce. IBS selectively focused on Lomant's Reagent, disclosed as having a disulfide bond that could be "quantitatively" cleaved using DTT at pH of 8.5. JA3017 ¶39; JA4935 at l.23-JA4936 at l.9; JA2970. Dr. Branchaud, however, conceded that DTT is a "sluggish" and inefficient cleaving agent at the appropriate pH of 7 for SBS applications. JA4925 at l.20-JA4926 at l.17; JA4931 at ll.3-21. Moreover, DNA has a negatively charged phosphate backbone that repulses the negatively charged reactive thiolate form of DTT. JA552-53; JA5152; JA3240. Thus, one of ordinary skill would not have expected the cleavage efficiencies of Pierce's protein cross-linking reagents to apply in the context of DNA.

efficiency after five separate washes with a cleavage reagent.[6] JA4117 at 11:12-17; JA47; JA50; JA3704 ¶¶57-58. After Herman was filed, several other publications reported extremely variable cleavage efficiencies using Herman's disulfide cleavage conditions. In 1989, Herman reported cleavage of "virtually all" of a disulfide linkage in "Bio-19-SS-dUTP," but provided no supporting data. JA2561; JA48. Two years later, Herman reported that the cleavage efficiency of a disulfide linkage in "Bio-19-SS-dUTP" was only "approximately one-half" and provided supporting data. JA5120; JA5124; JA48; JA552. An independent laboratory also reported that "reduction of the disulfide bond of Bio-19-SS-dUTP gave variable results" and did not rigorously pursue Herman's disulfide linkage. JA5131; JA49; JA552. The patent literature reported this same variability. JA5139 at 6:61-64.

The collective teachings of the Herman references and those using Herman's disulfide linker would have led one skilled in the art to have expected that conditions for cleavage of disulfide linkages would yield unpredictable cleavage efficiency. There was no reason based on these references for one skilled in the art to expect that conditions for cleaving a disulfide linkage in a nucleotide would yield greater than 90% efficiency. A skilled artisan would

[6] IBS attempted to inflate Herman's cleavage efficiency to 92.2% by making several unreliable assumptions and performing unreliable new calculations, JA462, but the Board recognized that Herman does not describe disulfide bond cleavage efficiency above 90%, JA47-48.

have expected the 3'-protecting group to cleave with no greater efficiency than the disulfide linkage, which the art demonstrated was below 90% and woefully insufficient for SBS. Accordingly, there would have been no motivation for one of skill to combine the selected features of the prior art to achieve Illumina's claimed invention. *See DePuy*, 567 F.3d at 1326 (*citing United States v. Adams*, 383 U.S. 39, 52 (1966)).

The Board's decision fails to provide any rationale for combining the prior art to arrive at the combination of a disulfide linkage attaching a label to the base and a 3'-protecting group that are cleavable under identical conditions as claimed by Illumina. *KSR*, 550 U.S. at 418 ("obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.") (*quoting In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)). Given Dr. Romesberg's unrebutted testimony, there was nothing before the Board evidencing an expectation that a 3'-protecting group would cleave under identical conditions with a disulfide linkage with greater than 90% efficiency. JA3701-05 ¶¶52, 55, 58, 60. Thus, those skilled in the art would have had no motivation to pursue a nucleotide containing a 3'-protecting group and a disulfide linkage that are cleavable under identical conditions. *Geo M. Martin Co. v. Alliance Mach. Sys. Int'l, LLC*, 618 F.3d 1294, 1303 (Fed. Cir.

2010) ("To be clear, prior art must teach a person of ordinary skill to make an apparatus that works for its intended purpose.").

When the substitute claims are considered in light of the evidence of record, there is no basis to combine the elements of the prior art to form Illumina's innovative nucleotides. It is only through impermissible hindsight that the Board combined prior art teachings to conclude that Illumina's substitute claims would have been obvious. *Ruiz*, 357 F.3d at 1275; *KSR*, 550 U.S. at 421.

d. The Board's conclusion that the disulfide linkage cleavage efficiency of the prior art could have been increased with an expectation of success was clearly erroneous

The Board erred in concluding that disulfide cleavage efficiency could have been increased with an expectation of success because: (1) the Board applied an incorrect standard for obviousness, and (2) the evidence demonstrates that the 3'-protecting group of a nucleotide having a 3'-protecting group and a disulfide linkage that cleave under identical conditions would not have been expected to cleave with sufficient efficiency for SBS.

i. The Board imposed an improperly heightened standard of nonobviousness

The Board imposed an improperly heightened standard of nonobviousness, requiring Illumina to show that it would not have been

possible to combine or modify the teachings of the prior art to arrive at the substitute claims. For example, the Board required Illumina to show that "the skilled worker would have been *unable* to choose conditions and linkages that would achieve 90% cleavage of the 3'-OH group under the same conditions required for cleavage of the label, e.g., using a reducing agent." JA44 (emphasis added). Similarly, the Board required Illumina to show that "efficiency yields above 90% *could not be achieved*." JA50 (emphasis added). The Board improperly required Illumina to "show that identical conditions *could not be selected* in which the disulfide linkage of the cleavable linker is cleavable with less than 90% efficiency and the protecting group is cleavable with greater than 90% efficiency as required by the proposed substitute claims." *Id.* (emphasis added). In essence, the Board required Illumina to prove that it would have been impossible to combine or modify the prior art to arrive at its claimed invention. This, however, is not the correct legal standard for obviousness. *See Procter & Gamble*, 566 F.3d at 994. The Board's failure to apply the appropriate standard was reversible error.

ii. <u>There is no evidence to support the Board's conclusion that a nucleotide having a disulfide linkage would provide sufficient cleavage efficiency of the 3'-protecting group</u>

No evidence supports the conclusion that the 3'-protecting group of a nucleotide having a 3'-protecting group and a disulfide linkage that are

cleavable under identical conditions would have been expected to cleave with greater than 90% efficiency, much less the 97-100% efficiency required for SBS. Yet the Board found that Ruby's 86% cleavage efficiency "could be modified to achieve the desired amount of cleavage." JA46. This finding is clearly erroneous. The Board improperly relied on Dr. Branchaud's unsupported and conclusory statement as its only support for this erroneous finding. *Id.* (*citing* JA3016 ¶37).

Dr. Branchaud asserted, without support, that cleavage efficiency could be improved by changing the cleavage agent, the concentration of the cleavage agent, the temperature, or the pH. JA3016 ¶37. But he provided no underlying facts or data supporting his opinion. *Id.* On the contrary, the record illustrates that Dr. Branchaud is incorrect. Ruby expressly states that it attempted to optimize the cleavage efficiency of a disulfide linkage using different pH values and various concentrations of the cleavage reagent DTT. JA4105-06. Yet, Ruby's most optimized results provided a maximum disulfide cleavage efficiency of only 86%. JA4105; *see also* JA3702-03 ¶54.

Dr. Branchaud relied upon Herman's 1989 publication as supposed support for an expectation of improved cleavage efficiency by lengthening the disulfide linker. *Id.* (*citing* JA2561). But in doing so, Dr. Branchaud omitted that just two years later Herman reported that only "approximately one-half" of

the disulfide linker was cleaved. JA5120. Dr. Branchaud also omitted that an independent laboratory did not rigorously pursue using this disulfide linker due to variable cleavage efficiency. JA5131; JA5139 at 6:61-64.

The widely variable and unpredictable results obtained with Herman's disulfide linkage cleavage conditions are insufficient to provide an expectation that a disulfide linkage and a 3'-protecting group that are cleavable under identical conditions would produce 3'-protecting group cleavage approaching 97-100% efficiency. Therefore, Dr. Branchaud's testimony regarding optimization was not supported by the prior art or sufficient underlying facts and data, and, accordingly, was entitled to little or no weight. 37 C.F.R. § 42.65(a) ("Expert testimony that does not disclose the underlying facts or data on which the opinion is based is entitled to little or no weight."). Moreover, the Board's apparent reasoning that because the prior art demonstrated variability a skilled artisan would have been able to optimize cleavage efficiency with a reasonable expectation of success is incorrect and unfounded.

Only Dr. Romesberg gave a reasoned, scientific opinion on the topic of optimizing the prior art cleavage conditions—and he testified that there was no expectation of successful improvement because the prior art references had already attempted to optimize the cleavage reaction efficiencies. JA2874 at l.24-JA2880 at l.20. As Dr. Romesberg explained during cross-examination,

modifications to Ruby's cleavage conditions would not have been expected to increase the cleavage efficiency because Ruby is "already at something of an optimal pH" (JA2875 at ll.4-10), the concentration of DTT is "already very high" (JA2876 at ll.12-15), and "a further increase in pH will buy you nothing, because you've already deprotonated the thiol" at the highest tested pH (JA2880 at ll.16-20).

Based on this reasoned analysis, Dr. Romesberg determined that the 86% cleavage efficiency reported by Ruby represents the "most efficient cleavage conditions after having optimized" the available parameters. JA2880 at ll.7-9. Even Dr. Branchaud conceded that attempts to vary Ruby's elution buffer resulted in a maximum cleavage efficiency of 86%. JA3014 ¶31. Yet the Board failed to adequately consider this testimony in finding that Ruby "could be modified to achieve the desired amount of cleavage." JA46. The evidence does not support the Board's conclusion. *In re Kao*, 639 F.3d 1057, 1067 (Fed. Cir. 2011) ("The Board has not provided any reason, apart from its own statement to the contrary, to question [the testimony of record]. The Board's own conjecture does not supply the requisite substantial *evidence* to support the rejections . . . ."); *In re Zeidler*, 682 F.2d 961, 967 (C.C.P.A. 1982). Therefore, the Board committed clear error in finding an expectation that it would have

been routine to modify the prior art cleavage efficiencies to achieve the levels required for SBS.

3. The Board did not identify any 3'-protecting group and disulfide linkage that are cleavable under identical conditions

"[O]bviousness requires a suggestion of all limitations in a claim." *CFMT, Inc. v. Yieldup Int'l. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (*citing In re Royka*, 490 F.2d 981, 985 (C.C.P.A. 1974)). An obviousness determination requires "a searching comparison of the claimed invention—including all its limitations—with the teaching of the prior art." *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995).

Illumina's substitute claims require a 3'-protecting group that is cleavable under identical conditions with the disulfide linkage attaching the label to the base. JA501-02. The Board, however, did not identify any particular 3'-protecting group in the prior art that would have been expected to meet this limitation. The Board erroneously stated that "Illumina provided evidence that the prior art teaches less than 90% efficiency in cleaving a disulfide linkage at [sic] 3'-OH protecting group . . . ." JA42. But this statement is incorrect, as none of the prior art before the Board contained a disulfide linkage at the **3'-OH protecting group**. JA510; JA512. Rather, the prior art only evidences disulfide linkages attached to the **base**. JA510-13. The Board's obviousness analysis was deficient in failing to identify any

3'-protecting group that would have been expected to cleave under identical conditions with a disulfide linkage attaching a label to the base.

IBS argued that a skilled artisan would choose a 3'-protecting group "from well-known protecting groups that would be cleavable under the same conditions as the disulfide linker." JA464-65. IBS's expert identified 2,4-dinitrobenzenesulfenyl and azidomethyl as such groups. JA3019-20 ¶¶48-49. IBS's expert, however, provided no motivation for selecting these two groups from the myriad of potential 3'-protecting groups and no basis for expecting success using these groups. IBS's expert conceded that the 2,4-dinitrobenzenesulfenyl group would not have been expected to be incorporated by the DNA polymerase used in SBS methods. JA4977 at ll.9-20. He also admitted that he used impermissible hindsight and selected the azidomethyl group based on an Illumina publication. JA4978 at l.9-JA4981 at l.22. IBS provided no evidence that a skilled artisan would have expected these groups to be incorporable by a polymerase or to cleave with sufficient efficiency for SBS. In contrast, Illumina provided evidence that the literature suggested otherwise. JA553-54.

The prior art does not provide an expectation that a 3'-protecting group that is cleavable under identical conditions with a disulfide linkage would cleave with sufficient efficiency for SBS. Accordingly, the Board's

obviousness determination cannot stand. *See KSR*, 550 U.S. at 418 (*quoting In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)).

The Board stated that "choosing cleavage conditions for the 3'-OH group were conventional to one of ordinary skill in the art" based on the specification of the '026 patent. JA44 (quoting JA69 at 8:28-34). The Board's reliance on this statement is misplaced. This statement is not an admission that it was well-established to use 3'-protecting groups that were cleavable under identical conditions as disulfide linkages. While 3'-protecting groups in general were conventional, none of the prior art references (in contrast to the '026 patent), alone or in combination, disclose or suggest a 3'-protecting group that is cleavable under identical conditions with a disulfide linkage, particularly in view of the stringent requirements for SBS. The Board erred by using this teaching of the specification as an admission that it was known in SBS to use a 3'-protecting group that is cleavable under identical conditions with a disulfide linkage. *See Heidelberger Druckmaschinen AG v. Hantscho Commercial Prods., Inc.*, 21 F.3d 1068, 1072 (Fed. Cir. 1994) ("The motivation to combine references can not come from the invention itself."). Therefore, Illumina's substitute claims would not have been obvious. *Lucent Techs., Inc. v. Gateway, Inc.*, 580 F.3d 1301, 1316 (Fed. Cir. 2009) (affirming nonobviousness where the cited reference was found "to be lacking at least one of the limitations" and

"no sufficient reason existed to modify the prior art" to arrive at the claimed invention); *Vizio, Inc. v. U.S. Int'l Trade Comm'n*, 605 F.3d 1330, 1343 (Fed. Cir. 2010).

4. The Board improperly discounted Illumina's evidence of unexpected results

Evidence of unexpected results must be considered as independent evidence of nonobviousness. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380 (Fed. Cir. 1986) (explaining that objective evidence such as unexpected results must be considered before reaching a conclusion on the issue of obviousness). Illumina presented testing showing that the claimed nucleotides demonstrated unexpected 3'-protecting group and disulfide cleavage efficiencies compared to the prior art. The Board erred by incorrectly determining that Illumina did not compare its claims to the closest prior art, JA52, and that Illumina's unexpected results were the mere recognition of the "latent properties" of the disulfide linkage, JA53-54.

a. Illumina presented testing demonstrating unexpected results using the claimed nucleotides

Illumina presented testing results showing that the claimed nucleotides demonstrated unexpectedly superior 3'-protecting group and disulfide linkage cleavage efficiencies under identical conditions and unexpectedly superior sequencing results. JA513-16; JA51. These experiments were detailed in the

Vermaas Declaration, JA4573-84 ¶¶6-19, and fully explained by Dr. Romesberg. JA3705-14 ¶¶61-74.

Illumina performed SBS using the claimed nucleotides, which specifically contained a disulfide linkage connecting a fluorescent label to the 7 position of 7-deazaadenosine and a 3'-azidomethyl protecting group (hereinafter the "dATP analog"). JA514; JA4575. The 3'-azidomethyl protecting group and the disulfide linkage are cleavable under identical conditions. JA514; JA3710 ¶67. The SBS experiments also used three other nucleotides: a dTTP analog, a dCTP analog, and a dGTP analog. JA514; JA4573-76 ¶6. The differences between these four nucleotides permitted identification of the incorporated nucleotide—A, T, C, or G—during the SBS experiments. JA514-15; JA3710-11 ¶¶68-70.

In each cycle of SBS, one of the four nucleotides is incorporated. JA514; JA3708 ¶63. Illumina measured fluorescence after incorporation. JA514; JA3708 ¶63. Next, the disulfide linkage of any incorporated dATP analog was cleaved with 2 mM of tris(hydroxymethyl)phosphine. JA3708-09 ¶64. Fluorescence was then measured a second time. JA3709 ¶65. Together, the two fluorescence measurements permitted identification of the incorporated nucleotide—A, T, C, or G—as follows: fluorescence in the first and second measurements indicated a dTTP analog was incorporated, fluorescence in only

the first measurement indicated a dATP analog was incorporated, fluorescence in only the second measurement indicated that a dCTP analog was incorporated, and no fluorescence in either measurement indicated that a dGTP analog was incorporated. JA3710-11 ¶¶68-69.

The success of these experiments for use in SBS depends on the efficient cleavage of the 3'-protecting group and the disulfide linkage attaching the label to the base on the nucleotides of Illumina's substitute claims (*i.e.*, the dATP analog) to accomplish multiple sequencing cycles. JA515-16; JA3711 ¶70; JA3713 ¶72. Illumina's experimental data demonstrates a high degree of sequencing accuracy with the nucleotides of the substitute claims, providing an error rate of just 0.43% over 150 cycles of sequencing. JA515; JA3713 ¶72. The low error rate indicates that the 3'-protecting group was cleaved with high efficiency in each round. JA3713 ¶72. The evidence also shows that the disulfide cleavage of the claimed nucleotides proceeds with greater than 99% efficiency. JA516; JA3713-14 ¶73. Moreover, this high disulfide cleavage efficiency occurs at an unexpectedly fast rate of about 23 seconds. JA516; JA3708 ¶62. The unexpectedly high cleavage efficiency of the 3'-protecting group and disulfide linkage that are cleavable under identical conditions and the surprisingly rapid cleavage rates are superior to the 86-87% cleavage

efficiencies and long cleavage times reported for the disulfide linkages in Ruby and Herman. JA516; JA3714 ¶74.

b. Illumina properly compared its claims to the closest prior art

In dismissing Illumina's evidence of unexpected results, the Board incorrectly concluded that "Illumina does not state what references are the closest prior art to the claims." JA52. Illumina, however, explicitly stated in its Motion to Amend: "The closest prior art discloses nucleotides for sequencing by synthesis, but fails to teach or suggest a nucleotide triphosphate molecule having a 'disulfide linkage' in the cleavable linker or cleavage of a disulfide linkage and protecting group under identical conditions." JA508. Illumina systematically compared the disclosure of the prior art references with the substitute claims. JA508-13; JA3697-705 ¶¶45-60. This comparison identified Ruby and Herman as the only prior art references that disclosed numerical cleavage efficiency data for disulfide linkages. JA511. Therefore, Ruby and Herman constitute the appropriate closest prior art benchmark comparator for assessing the efficiency that a skilled artisan would have expected for cleaving a disulfide linkage, and in turn the 3'-protecting group, in the nucleotides of Illumina's substitute claims. *See In re Merchant*, 575 F.2d 865, 868 (C.C.P.A. 1978) ("A comparison of the *claimed* invention with the disclosure of each cited reference to determine the number of claim limitations in common with

each reference, bearing in mind the relative importance of particular limitations, will usually yield the closest single prior art reference.").

The Board was apparently dissatisfied with the use of Ruby and Herman as the prior art comparators because "the references do not use the nucleotide in a sequencing reaction as it has been used in the experiment described by Mr. Vermaas." JA52. It is telling that the only prior art reporting numerical cleavage data for disulfide linkages (Ruby and Herman) are directed to applications far afield from SBS. Moreover, neither of these references (nor any cited reference) provides any data for the cleavage efficiency of a 3'-protecting group that is cleavable under identical conditions as a disulfide linkage.

The Board further faulted Illumina for conducting SBS testing with tris(hydroxymethyl)phosphine as the cleavage agent, instead of the DTT used by Ruby and Herman. JA53. This criticism is misplaced. Illumina's testing was conducted in the ordinary course of product development, before IBS even filed its petition. JA798 at ll.1-12; JA2507-13 (showing dates of Illumina's testing). The fact that Illumina's pre-IPR product development work did not use the same cleavage agent as Ruby and Herman does not preclude the comparison submitted by Illumina, as direct comparison testing is not required to demonstrate unexpectedly superior results. *See In re Merchant*, 575 F.2d at

869 n.8; *see also In re Blondel*, 499 F.2d 1311, 1317 (C.C.P.A. 1974). Illumina's testing demonstrated disulfide cleavage efficiency of greater than 99%, which is unexpectedly superior to the results of 86-87% (or variable) efficiency expected based on Ruby and Herman. JA516; JA3714 ¶74. These results are sufficient to support the nonobviousness of the substitute claims. *See In re Fouche*, 439 F.2d 1237, 1241 (C.C.P.A. 1971) (concluding that indirect evidence of unexpected superiority is sufficient to show nonobviousness).

c. Illumina demonstrated that the unexpected results are not a latent property of the disulfide linkage

The Board improperly dismissed Illumina's evidence of unexpected results as "'the mere recognition' of the [disulfide] bond's 'latent properties'." JA53-54. Latent properties cannot be used to show nonobviousness of a *known* compound. *In re Baxter Travenol Labs*, 952 F.2d 388, 392 (Fed. Cir. 1991). Illumina's claimed nucleotides, and particularly the combined elements of a disulfide linkage attaching a label to the base and a 3'-protecting group cleavable under identical conditions, were completely *unknown* in the prior art. JA512-13; JA3680 ¶1. When a claimed compound is unknown in the prior art, evidence of unexpected results must be considered. *In re Sullivan*, 498 F.3d 1345, 1353 (Fed. Cir. 2007). As such, Illumina's evidence of unexpected results "cannot be ignored." *Id.* (finding that the Board erred, as a matter of law, by failing to consider evidence of unexpected results for an unknown

compound); *see also In re Mills*, 916 F.2d 680, 683 (Fed. Cir. 1990). The Board erred by failing to consider Illumina's evidence of unexpected results. *In re Soni*, 54 F.3d 746, 751 (Fed. Cir. 1995) ("when an applicant demonstrates *substantially* improved results, as Soni did here, and *states* that the results were *unexpected*, this should suffice to establish unexpected results *in the absence of* evidence to the contrary") .

5. Illumina demonstrated the patentability of the substitute claims

Illumina's Motion to Amend fully complied with the statutory and regulatory requirements for such motions. *See* 35 U.S.C. § 316(d); 37 C.F.R. § 42.121. Illumina submitted four substitute claims to replace cancelled Claims 1-8. JA501; *see also* 35 U.S.C. § 316(d)(1); 37 C.F.R. § 42.121(a)(3). The substitute claims are narrower in scope than the issued claims and are fully supported by the specification. JA503-05; JA3685-93 ¶¶26-33; *see also* 35 U.S.C. § 316(d)(3); 37 C.F.R. § 42.121(a)(2)(ii); 37 C.F.R. § 42.121(b)(1). The substitute claims also rendered moot each of the grounds upon which the Board instituted trial. JA500-01; *see also* 37 C.F.R. § 42.121(a)(2)(i). There is no dispute that Illumina's Motion to Amend satisfied all procedural requirements.

Illumina also explained in detail why substitute Claims 9-12 are patentable over the prior art. Accordingly, Illumina met the requirements of the

controlling statutes and regulations and demonstrated the patentability of the substitute claims.

VI. CONCLUSION

The Board erred in denying Illumina's Motion to Amend the '026 patent to add substitute Claims 9-12 and in concluding that the substitute claims would have been obvious. Illumina respectfully requests that this Court reverse the Board's erroneous decision and grant Illumina's Motion to Amend, or, alternatively, vacate the Board's erroneous decision and remand for proper consideration of Illumina's Motion to Amend.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: March 10, 2015

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ADDENDUM

Table to Addendum

Trials@uspto.gov
571-272-7822

Paper 92
Entered: July 25, 2014

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTELLIGENT BIO-SYSTEMS, INC.,
Petitioner,

v.

ILLUMINA CAMBRIDGE LIMITED,
Patent Owner.

Case IPR2013-00128
U.S. Patent 7,057,026 B2

Before LORA M. GREEN, RICHARD M. LEBOVITZ, and CHRISTOPHER L. CRUMBLEY, *Administrative Patent Judges*.

LEBOVITZ, *Administrative Patent Judge*.

FINAL WRITTEN DECISION
35 U.S.C. § 318(a) and 37 C.F.R. § 42.73

I. BACKGROUND

A. Introduction

Petitioner, Intelligent Bio-Systems, Inc. ("IBS"), filed a Petition (Paper 15, "Pet.") for *inter partes* review of claims 1-8 of U.S. Patent No. 7,057,026 B2 ("the '026 patent") pursuant to 35 U.S.C. §§ 311-319 and 37 C.F.R. §§ 42.1–42.123.

On July 29, 2013, *inter partes* review of claims 1-8 was instituted on five grounds of unpatentability. Decision on Petition ("Dec. Pet.") (Paper 23). After institution of the *inter partes* review, Patent Owner, Illumina Cambridge Limited ("Illumina"), did not file a response under 37 C.F.R. § 42.120 to the decision instituting *inter partes* review.

Illumina filed a Motion to Amend (Paper 61, "Mot. Amend") and a Motion to Exclude Evidence (Paper 70). IBS filed an opposition to Illumina's Motion to Amend (Paper 53) and its own Motion to Exclude Evidence (Paper 67). An oral hearing was held on April 23, 2014. Record of Oral Hearing (Paper 89).

The Board has jurisdiction under 35 U.S.C. § 6(c). This final written decision is issued pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73.

Illumina's Motion to Amend is GRANTED to the extent it requests to cancel claims 1-8; Illumina's Motion to Amend is DENIED to the extent that it requests entry of substitute claims 9-12.

B. The '026 Patent

The '026 patent issued on June 6, 2006. The named inventors are Colin Barnes, Shankar Balasubramanian, Xiaohai Liu, Harold Swerdlow, and John Milton. The '026 patent describes labeled nucleotides and

nucleosides used in "sequencing reactions, polynucleotide synthesis, nucleic acid amplification, nucleic acid hybridization assays, single nucleotide polymorphism studies, and other techniques using enzymes such as polymerases, reverse transcriptases, terminal transferases, or other DNA modifying enzymes." Ex. 1001, col. 2, ll. 10-14. A detectable label (such as a fluorophore) is attached to the base of the nucleotide via a cleavable linker group. *Id.* at col. 2, ll. 6-8. In DNA sequencing by synthesis ("SBS"), nucleotides are added sequentially to a newly synthesized DNA strand complementary to the template DNA in the double-stranded DNA. The detectable label enables the nucleotide to be detected when it is incorporated into the newly synthesized DNA strand. *Id.* at col. 2, ll. 56-64. Once the identity of the nucleotide is determined by detecting the label linked to the base, the detectable label is cleaved from the nucleotide by the cleavable linker. *Id.* at col. 2, ll. 60-64, col. 6, ll. 26-30. The 3′-OH of the sugar residue of the nucleotide contains a protecting group, which can be removed to expose the 3′-OH group for further addition of a nucleotide. *Id.* at col. 2, ll. 30-32, col. 8, ll. 8-15.

C. Related Proceedings

The '026 patent is asserted in the following copending district court case: *Trustees of Columbia University in the City of New York v. Illumina, Inc.*, 1:12-cv-00376-GMS in the United States District Court for the District of Delaware. Pet. 1.

D. The Alleged Grounds of Unpatentability

Inter partes review was instituted on the following five grounds of unpatentability (Dec. Pet. 18):

1. Claims 1-6 under 35 U.S.C. § 102(b) as anticipated by Tsien.[1]

2. Claims 1-6 under 35 U.S.C. §§ 102(a) or 102(e) as anticipated by Ju.[2]

3. Claim 3 under 35 U.S.C. § 103(a) as obvious in view of Tsien and Prober.[3]

4. Claims 7 and 8 under 35 U.S.C. § 103(a) as obvious in view of Tsien and CEQ.[4]

5. Claims 7 and 8 under 35 U.S.C. § 103(a) as obvious in view of Ju and CEQ.

II. CLAIMS 1-8

Illumina did not file a response to the Decision on Petition instituting *inter partes* review of claims 1-8. Instead, Illumina filed a non-contingent Motion to Amend. Paper 61. "During an inter partes review . . . the patent owner may file 1 motion to amend the patent in 1 or more of the following ways: (A) Cancel any challenged patent claim. (B) For each challenged claim, propose a reasonable number of substitute claims." 35 U.S.C. § 316(d)(1). In the Motion, Illumina requested cancellation of claims 1-8, which request was not contingent on the original claims being determined unpatentable, and proposed substitute claims 9-12 to replace the cancelled claims. Illumina stated that each of the grounds upon which the *inter partes*

[1] Tsien, WO 91/06678 (published May 16, 1991). Ex. 1012.
[2] Ju, U.S. 6,664,079 B2 (published Dec. 16, 2003). Ex. 1008.
[3] Prober et al., "A System for Rapid DNA Sequencing with Fluorescent Chain-Terminating Dideoxynucleotides." 238 SCIENCE 336 (Oct. 16, 1987). Ex. 1013.
[4] CEQ™ User's Guide, Beckman Coulter CEQ™ 2000 DNA Analysis System User's Guide (June 2000). Ex. 1006.

review was instituted "is rendered moot in light of Illumina's proposed substitute claims." Paper 61, 2. We shall GRANT Illumina's Motion to Amend to the extent it requests to cancel claims 1-8.

III. MOTION TO AMEND THE CLAIMS

In the Motion to Amend, Illumina proposed substitute claim 9 to replace claim 1. The claim, as annotated by Illumina to show the differences between original claim 1 and proposed substitute claim 9, is reproduced below:

> 9. A nucleotide triphosphate ~~or nucleoside~~ molecule, having a 7-deazapurine base that is linked to a detectable label via a cleavable linker, wherein the cleavable linker is attached to the 7-position of the 7-deazapurine base and wherein the cleavable linker contains a disulfide linkage, and wherein the nucleotide triphosphate molecule has a ribose or deoxyribose sugar moiety comprising a protecting group attached via the ~~2′ or~~ 3′ oxygen atom, and the disulfide linkage of the cleavable linker and the protecting group are cleavable under identical conditions.

Paper 61, 2.

Proposed substitute claim 9 requires a nucleotide having a 1) triphosphate group; 2) a deazapurine base; 3) a disulfide linkage as a cleavable linker; and 4) a protecting group on the 3′ oxygen. Original claim 3, now canceled, recited that the base of the nucleotide is a deazapurine as recited in proposed substitute claim 9. This limitation, therefore, had been considered in the Decision on Petition. None of the original claims, however, comprised the limitation that the cleavable linker "contains a disulfide linkage." The obviousness of using a disulfide linkage is the main issue to be decided in whether to grant the Motion to Amend.

Proposed substitute dependent claims 10 and 12 replace claims 5 and 8, respectively. Proposed substitute independent claim 11 replaces claim 7, and recites a nucleotide with the same features of claim 9.

Patent Owner bears the burden of proof to establish that it is entitled to the relief requested in the Motion to Amend. 37 C.F.R. § 42.20(c). Patent Owner, therefore, bears the burden of showing the patentability of the amended claims.

Patent Owner must show that the conditions for novelty and non-obviousness are met for the prior art available to one of ordinary skill in the art at the time the invention was filed, not just the prior art cited in the Petition. *See Idle Free Sys., Inc. v. Bergstrom, Inc.*, Case IPR 2012-00027, slip op. at 7 (PTAB June 11, 2013) (Paper 26). Also, a motion to amend "must include a claim listing, show the changes clearly, and set forth: (1) The support in the original disclosure of the patent for each claim that is added or amended." 37 C.F.R. § 42.121(b)(1).

IV. PATENTABILITY OF CLAIMS 9-12

A. Background

The '026 patent relates to labeled nucleotides. For illustrative purposes, an annotated generic nucleotide from Figure 1B of Stemple,[5] one of the prior art publications cited in this *inter partes* review, is reproduced below to show the nucleotide's main parts.

[5] Stemple, WO 00/53805 (published Sep. 14, 2000). Ex. 1002.

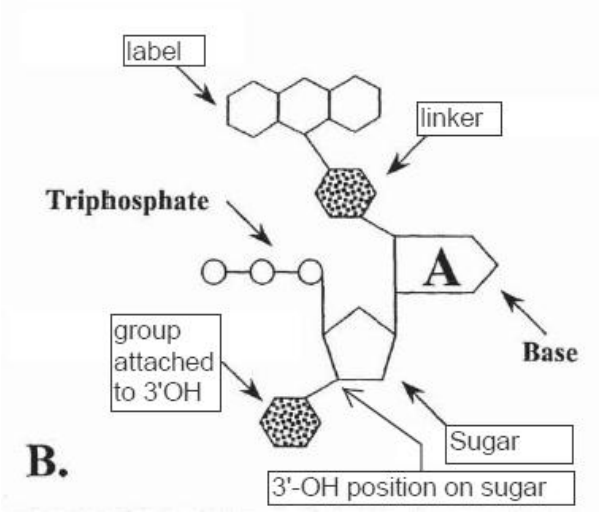


Figure 1B of Stemple shows a generic nucleotide's main parts, including a detectable label ("label"), a linker, and a chemical group attached to the 3′-OH position.

The '026 patent describes its invention as nucleotide molecule "linked to a detectable label via a cleavable linker group attached to the base." Ex. 1001, col. 2, ll. 6-8. The figure reproduced above shows a single ring-like structure, labeled "linker," which links a three-ringed structure, labeled "label," to the base. The '026 patent discloses that the sugar "can include a protecting group attached via the 2' or 3' oxygen atom. The protecting group can be removed to expose a 3′-OH." *Id.* at col. 2, ll. 30-33. The figure shows a protecting group attached to the 3′-OH of the sugar molecule.

Original claim 1 was drawn to a nucleotide or nucleoside with the following features:

1. "a base that is linked to a detectable label via a cleavable linker" (see "label," "linker," and "base" in Figure 1B of Stemple reproduced above);

2. "a ribose or deoxyribose sugar moiety comprising a protecting group attached via the 2′ or 3′ oxygen atom" (the figure shown above shows a protecting group attached to the oxygen of the 3′-OH group); and

3. "the cleavable linker and the protecting group are cleavable under identical conditions."

Proposed substitute claim 9 contains these features, but further recites that the linker comprises a disulfide linkage (two sulfur atoms bonded together) and that the nucleotide base is a deazapurine.

B. Claim Interpretation

Before a claim can be compared to the prior art, it must be interpreted. In an *inter partes* review, claim terms in an unexpired patent are given their broadest reasonable interpretation consistent with the specification of the patent in which they appear. 37 C.F.R. § 42.100(b). Under the broadest reasonable interpretation standard, claim terms are given their ordinary and customary meaning as they would be understood by one of ordinary skill in the art at the time of the invention. *In re Morris*, 127 F.3d 1048, 1054 (Fed. Cir. 1997).

1. *deazapurine*

A deazapurine, as recited in proposed substitute claim 9, is interpreted to mean a nitrogen base in which one of the natural nitrogen atoms in the base ring is substituted with a carbon atom. Ex. 1008, col. 8, ll. 4-6.

2. *the cleavable linker and the protecting group are cleavable under identical conditions*

The proposed claim requires a "cleavable linker," which is used to attach the "detectable label" to the 7-position of the deazapurine base. The reason for having a cleavable linker at this position is explained as follows.

In sequencing by synthesis reactions ("SBS"), nucleotides are added sequentially, one at a time. Ex. 1001, col. 2, ll. 56-61. The "identity of each nucleotide incorporated is determined by detection of the label linked to the base, and subsequent removal of the label." *Id.* at col. 2, ll. 61-64. The base is removed by cleaving the disulfide linkage of the cleavable linker ("the disulfide linkage of the cleavable linker [is] . . . cleavable"). *Id.* at Claim 9. The claimed "cleavable linker" is, therefore, necessary to carry out SBS sequencing.

The 3′ oxygen, labeled as the "3′ OH of the sugar" in the figure reproduced above, is recited in proposed substitute claim 9 to comprise a "protecting group." The "protecting group" is described in the '026 patent as "intended to prevent nucleotide incorporation onto a nascent polynucleotide strand." Ex. 1001, col. 8, ll. 11-12. It can be removed by cleavage to allow sequential addition of a nucleotide during SBS sequencing. *Id.* at col. 8, ll. 12-14.

The claims do not specify the type of protecting group. The '026 patent teaches at column 8, lines 28-31, "[s]uitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in Green and Wuts, supra. Some examples of such protecting groups are shown in FIG. 3."

Proposed substitute claim 9 recites that "the cleavable linker and the protecting group are cleavable under identical conditions." The '026 patent discloses that the "labile linker may consist of functionality cleavable under identical conditions to the block [the protecting group]. This will make the deprotection process [of the 3′-OH group] more efficient as only a single treatment will be required to cleave both the label and the block." Ex. 1001,

col. 8, ll. 35-44. Thus, "cleavable under the identical conditions," under the broadest reasonable interpretation standard, limits the structure of the protecting group to one which can be cleaved under the same conditions as a disulfide linkage, but does not require a specific structure, such as a disulfide linkage. *See* Paper 61, 6-7.

3. *cleavable linker contains a disulfide linkage*

The main limitation at issue in Illumina's Motion to Amend is the recitation in all the proposed substitute claims that a 7-deazapurine base is linked to a detectable label through a "cleavable linker [which] contains a disulfide linkage." A disulfide linkage is a bond between two sulfur atoms. Ex. 1001, Fig. 2.

C. Illumina's Burden to Show Nonobviousness

The issue in this *inter partes* review is the obviousness of using a cleavable disulfide linker to attach a label to the nucleotide base, where the linker and protecting group of the nucleotide are cleaved under identical conditions.

Because Illumina bears the burden of showing that it is entitled to have its Motion to Amend granted, it must show that one of ordinary skill in the art would not have considered the proposed substitute claims obvious in view of the prior art available before the filing date of the claimed invention. More specifically, the issue is whether it would have been nonobvious at the time of the invention to have attached a detectable label to a deazapurine base using a disulfide linkage, where the base is present in a nucleotide triphosphate having "a ribose or deoxyribose sugar moiety comprising a

protecting group attached via the 2′ or 3′ oxygen atom" and where "the disulfide linkage of the cleavable linker and the protecting group are cleavable under identical conditions." Proposed claim 9.

A patent claim is invalid for obviousness if "the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains." 35 U.S.C. § 103.

> [The] underlying factual considerations in an obviousness analysis include the scope and content of the prior art, the differences between the prior art and the claimed invention, the level of ordinary skill in the art, and any relevant secondary considerations. Relevant secondary considerations include commercial success, long-felt but unsolved needs, failure of others, and unexpected results.

Allergan, Inc. v. Sandoz Inc., 726 F.3d 1286, 1291 (Fed. Cir. 2013).

An important consideration is "whether a person of ordinary skill in the art would, at the relevant time, have had a 'reasonable expectation of success' in pursuing the possibility that turns out to succeed and is claimed." *Institute Pasteur & Universite Pierre et Marie Curie v. Focarino*, 738 F.3d 1337, 1344 (Fed. Cir. 2013).

D. Prior Art

Before turning to the specific arguments presented by both parties, we shall summarize some of the prior art of record that was known at the time the application resulting in the '026 patent was filed on August 23, 2002. This discussion is not meant to be exhaustive, but rather provides a brief description of what was known about modified nucleotides prior to the filing date of the '026 patent.

The claimed nucleotides are used in nucleic acid SBS, a process in which 3′-OH protected and detectably labeled nucleotides are added stepwise to a nucleic acid primer during sequencing. In that process, it was known to use a nucleotide labeled at its base with a detectable label in order to identify when the nucleotide is incorporated into the newly synthesized strand. Ex. 1012, p. 27, l. 33-p. 28, l. 2; Ex. 1014,[6] col. 18, l. 64–col. 19, l. 2. It also was known to attach a protecting group to the 3′-OH of the nucleotide. Ex. 1012, p. 9, l. 32-p. 10, l. 3. During DNA synthesis, nucleotides are added sequentially to the 3′-OH group of the nucleotide sugar. The 3′-OH group contains a removable protecting group so the labeled nucleotides can be added one at a time. After each addition, the label is detected and the 3′-OH group is deblocked and new nucleotide (with its own 3′-OH protecting group) is added. *Id.* at 13. In sum, it was not new to employ a nucleotide in sequencing which comprised a detectable label on the nucleotide base and a 3′-OH protecting group.

The prior art also had described attaching a label to the nucleotide base using a cleavable linker as recited in the proposed claims. Ex.1012, p. 28, ll. 20-23; Ex. 1008, Abstract, col. 2, ll. 50-53. Furthermore, as found in the Decision on Petition, Tsien described removing the detectable label and the protecting group simultaneously, as required by the proposed claims. Ex.1012, p. 28, ll. 5-8; Dec. Pet. 8.

The newly added limitation that the cleavable linker is a disulfide bond also is described in the prior art. As discussed in more detail below, Rabani[7] and Church[8] both describe attaching a detectable label to a

[6] Dower, W.J. & Fodor, S.P.A. U.S. 5,547,839, issued Aug. 20. 1996.
[7] Rabani, E. WO 96/27025 (published Sept. 6, 1996). Ex. 2015.

nucleotide base via a disulfide linkage, where the nucleotide is used in nucleic acid sequencing. An example is shown in Figure 5 of Church, reproduced below, which we have annotated to identify specific structures.

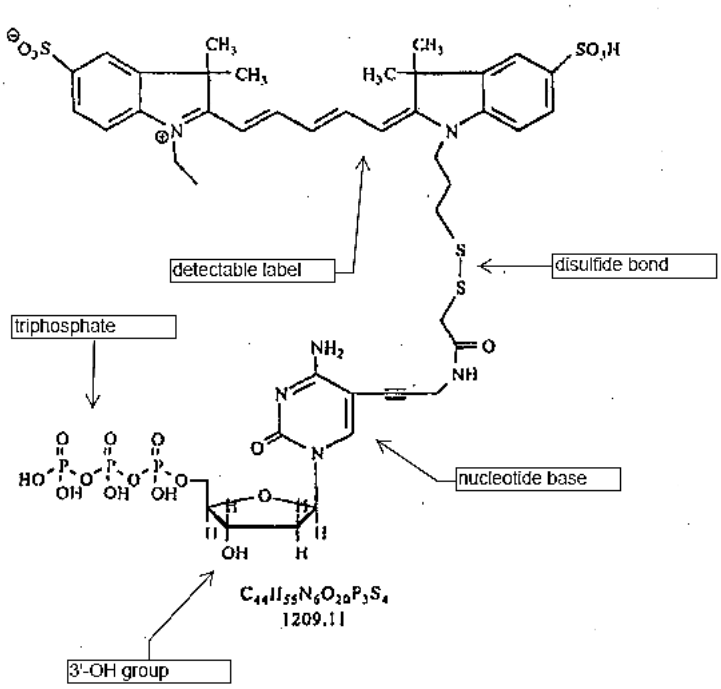
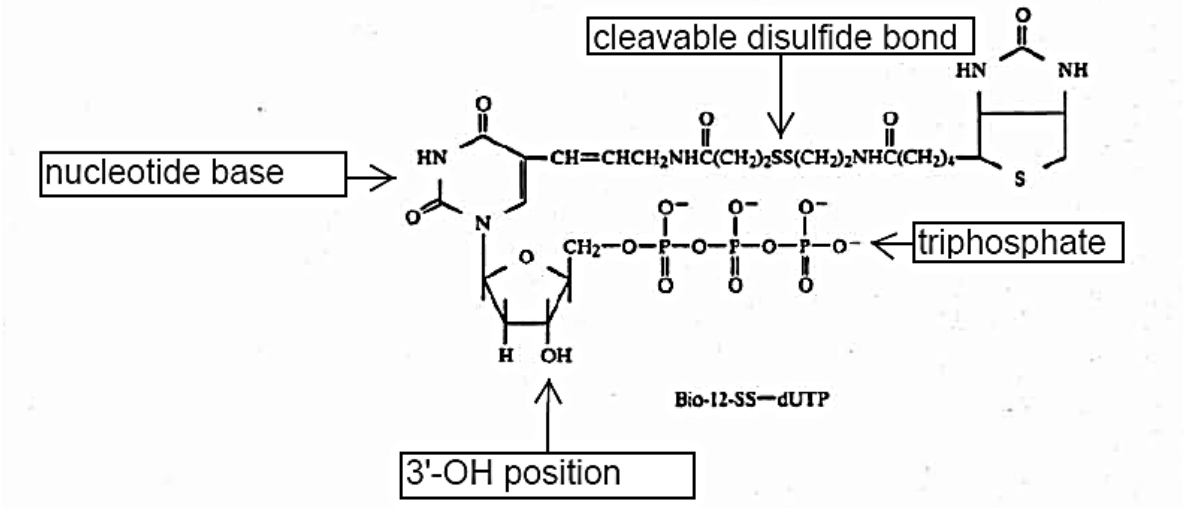


Figure 5 of Church is annotated to identify the specific structures of the nucleotide, including a detectable label, a triphosphate, and a disulfide bond. Ex. 1031, p. 17, ll. 10-11 ("Figure 5 is a schematic drawing of a disulfide-bonded cleavable nucleotide fluorophore complex."). The nucleotide, however, lacks the claimed protecting group on the 3′-OH.

In addition to Church, six additional publications[9] are cited in this *inter partes* review for their description of nucleotides comprising cleavable

[8] Church, G.M. WO 00/53812 (published Sept. 14, 2000). Ex. 1031.
[9] (1) Herman, US Patent No. 4,772, 691, issued Sep. 20, 1988. Ex. 2017.
(2) S.W. Ruby, et al., *Affinity Chromatography with Biotinylated RNAs*, Vol. 181, Methods in Enzymology, 97-121 (1990). Ex. 2016.
(3) Short, WO 99/49082 (published Sep. 30, 1999). Ex. 2018.

linkers with disulfide bonds. One of these publications, Herman, shows a nucleotide with disulfide bond attaching a biotin to a nucleotide base. Exhibit 2017. The orientation of the nucleotide reproduced below is flipped 180 degrees from Church's nucleotide, reproduced above.



Herman's Figure (col. 5) shows a nucleotide with a cleavable linker comprising a disulfide bond joining a biotin ("detectable label") to a nucleotide base. Ex. 2017, col. 7, ll. 24-27. The nucleotide lacks the protecting group on the 3′-OH as required in proposed claim 9.

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(4) Barbara A. Dawson, et al., *Affinity Isolation of Transcriptionally Active Murine Erythroleukemia Cell DNA Using a Cleavable Biotinylated Nucleotide Analog*, Vol. 264, No. 22, The Journal of Biological Chemistry, 12830-37 (1989). Ex. 1030.
(5) Barbara A. Dawson, et al., *Affinity Isolation of Active Murine Erythroleukemia Cell Chromatin: Uniform Distribution of Ubiquitinated Histone H2A Between Active and Inactive Fractions*, Vol. 46, Journal of Cellular Biochemistry, 166-173 (1991). Ex. 2039.
(6) Basil Rigas, et al., *Rapid plasmid library screening using RecA-coated biotinylated probes*, Vol. 83, Proc. Natl. Acad. Sci. USA, 9591-9595 (1986). Ex. 2040.

E. Reason to Use a Disulfide Bond on Nucleotides

In the Decision on Petition, *inter partes* review was authorized for claim 3 (reciting that the nucleotide base is a deazapurine) as anticipated under 35 U.S.C. § 102 in view of Tsien (Ground I) and Ju (Ground II); and as obvious under 35 U.S.C. § 103 in view of Tsien and Prober (Ground IV). Those publications, however, do not describe using a cleavable disulfide linker for attaching the detectable label to a base or the specific deazapurine base recited in the proposed substitute claims. A disulfide bond as a linker is described in the prior art (see pages 12-13 above), along with a reason to have used one.

Rabani

Rabani, in the section titled "Cleavable linkers," teaches that "[l]abeling moieties are favorably in communication with or coupled to nucleotides via a linker of sufficient length to ensure that the presence of said labeling moieties on said nucleotides will not interfere with the action of a polymerase enzyme on said nucleotides." Ex. 2015, p. 32, ll. 10-13. Rabani specifically mentions disulfide linkages as useful when a cleavable linker is desired:

> Linkages comprising disulfide bonds within their length have been developed to provide for cleavability[24]; reagents comprising such linkages are commercially available[25] and have been used to modify nucleotides[26] in a manner which may be conveniently reversed by treatment with mild reducing agents such as dithiothreitol.

Id. at p. 32, ll. 29-33. Footnotes 24 and 26 of the above quoted passage cite to Ruby, S.W. et al. (1990), Methods in Enzymology, 181:97, which is

Exhibit 2016. *Id.* at 49. Footnote 25 references "for example, from Pierce Chemical Co., [ ] of Rockford, IL., U.S.A." *Id.*

Rabani, therefore, would have given a skilled worker reason to have used a cleavable linker with a disulfide bond to ensure that the labeling moieties on the nucleotides will not interfere with the action of a polymerase enzyme during the synthesis reaction.

Church

IBS cited Church as evidence of the obviousness of using a disulfide linker in a sequencing reaction. Paper 53, 2. Church provides another example of the use of a disulfide linker to attach a label to base of a nucleotide, further establishing its conventionality at the time of the invention. Ex. 1031, 17:10-18, 68:12-21. Church describes a working example in which a label was attached to a nucleotide base using a disulfide linker and then cleaving it off with DTT. *Id.* at 86:6-30.

Dr. Bruce P. Branchaud,[10] a declarant for IBS, testified:

> Church teaches a SBS [sequencing by synthesis] method termed fluorescent in situ sequencing extension quantification (FISSEQ). In one embodiment, Church teaches the sequential addition of fluorescently labeled nucleotides in which the label

[10] To support its obviousness challenge, IBS provided two Declarations by Bruce P. Branchaud, Ph.D., Ex. 1015 and Ex. 1035, respectively. Dr. Branchaud is Professor Emeritus in the Department of Chemistry at the University of Oregon. Ex. 1015 ¶ 5. He has a Ph.D. in Organic Chemistry from Harvard University, and has held positions in industry, including as an internal consultant and advisor for DNA sequencing projects. Ex. 1015 ¶¶ 5, 7, 12-15. Dr. Branchaud has the requisite familiarity with DNA sequencing to qualify as one of ordinary skill in the art at the time of the invention. Consequently, we conclude that Dr. Branchaud is qualified to testify on the matters addressed in his Declaration.

> is attached to the base via a "cleavable linkage". Church at p. 67, l. 30 to p. 68, l. 11.

Ex. 1035 ¶ 13.

F. 90% Cleavage Efficiency of the Detectable Label Is Not Required

Illumina contends sequencing by synthesis processes require that the disulfide linker joined to the detectable label be cleaved with 90% efficiency, because of the iterative nature of the process. Paper 61, 11-12. This reasoning is based on Rabani. *Id.* Given Rabani's disclosure, as discussed below, Illumina argues that a person of ordinary skill in the art would not have used a disulfide bond because greater than 90% efficiency in the cleavage reaction could not be achieved.

Rabani disclosed published results "suggest[ing] that the rate of chemical removal of 3'-hydroxy protecting groups (less than 90% removal after 10 minutes of treatment with 0.1M NaOH) will be unacceptably low for such an inherently serial sequencing scheme." Ex. 2015, 3:5-8 (emphasis added). Illumina contends that since Rabani teaches that less than 90% removal of the protecting group from the 3′-OH "will be unacceptably low for . . . [a] serial sequencing scheme," and since "the disulfide linkage of the cleavable linker and the protecting group are cleavable under identical conditions," the disulfide linker joining the detectable label to the nucleotide base must also achieve 90% or more cleavage. Paper 61, 13; Ex. 2015, 3:5-8. Illumina provided evidence that the prior art teaches less than 90% efficiency in cleaving a disulfide linkage at 3′-OH protecting group, leading Illumina to reason "the expectation of unacceptably low 3′-OH protecting group cleavage efficiency when using 'identical conditions' would not lead a skilled artisan to believe that one successfully could use 3′-OH protecting

groups that could be cleaved under identical conditions as a disulfide linkage during SBS [sequencing by synthesis]." Paper 61, 13.

In other words, Illumina's argument is that since 90% efficiency in cleaving the disulfide linkage at the 3′-OH group could not be achieved, there would not have been a reason to use a disulfide linkage to attach the detectable label to the base, because the detectable label must cleaved under identical conditions to the 3′-OH protecting group. Cleavage of the disulfide linkage at the 3′-OH group requires 90% efficiency Illumina argues 90% cleavage efficiency must be achieved at the disulfide bond of the detectable label, as well.

Illumina's argument is flawed. Rabani's disclosure is directed to cleavage of the protecting groups, not the detectable label as claimed. Illumina's arguments are based on the logic that if no better than 90% cleavage of the disulfide bond on the protecting group can be achieved, the skilled worker would not have used it as a cleavable linker for attaching a detectable label to a nucleotide in DNA sequencing, because the proposed substitute claims require it be cleaved under identical conditions to the 3′-OH group, which requires 90% efficiency.

The proposed substitute claims do not require the linkage between the 3′-OH and protecting group to comprise a disulfide bond. We, therefore, discern no reason to apply Rabani's protecting group cleavage efficiency requirement to the disulfide linkage of the proposed substitute claims.

Significantly, Dr. Floyd Romesberg,[11] a declarant for Illumina, conceded that he did not choose a 90% efficiency requirement because of

[11] A declaration by Floyd Romesberg, Ph.D. was submitted by Illumina in support of its Motion to Amend. Ex. 2009. Dr. Romesberg is a professor in

Rabani, but rather because "it was a round number slightly above the values reported by Ruby and Herman." Ex. 1033, 198, ll.11-18.

Illumina has not met its burden to show that cleavage of the disulfide bond, attaching the detectable label to the base, with less than 90% efficiency would be unacceptable for sequencing.

G. Cleavage Efficiency of the Disulfide Bond

Even were we persuaded by Illumina's argument that 90% cleavage of the 3′-OH protecting group is necessary for sequencing, Illumina did not provide adequate evidence that the skilled worker would have been unable to choose conditions and linkages that would achieve 90% cleavage of the 3′-OH group under the same conditions required for cleavage of the label, e.g., using a reducing agent (Ex. 1001, col. 6, ll. 31-35).

The '026 patent suggests that choosing cleavage conditions for the 3′-OH group were conventional to one of ordinary skill in the art:

> Suitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in Green and Wuts, supra. Some examples of such protecting groups are shown in FIG. 3. The protecting group should be removable (or modifiable) to produce a 3' OH group. The process used to obtain the 3' OH group can be any suitable chemical or enzymic reaction.

Ex. 1001, col. 8, ll. 28-34.

Dr. Branchaud testified that "even if one of ordinary skill in the art considered the cleavage efficiency of *Ruby* . . . in deciding whether to use

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the Department of Chemistry at The Scripps Research Institute, where he has been a faculty member since 1998. Ex. 2009 ¶ 2. Dr. Romesberg testified that he is an "expert in the field of nucleotide analogue molecules." *Id*. at ¶ 16. Dr. Romesberg's deposition is Exhibit 1033.

such a linker for SBS, one of ordinary skill in the art would know that such cleavage efficiency could be improved by routine experimentation and thus would not be dissuaded from using such a linker." Ex. 1035 ¶ 37. As discussed below, this conclusion is supported by the prior art of record.

Ruby

Ruby is cited expressly by Rabani for its teaching of a disulfide bond that is cleavable with a reducing agent, such as dithiothreitol ("DTT"), and describes attaching a biotin molecule attached to a nucleotide base of a RNA "via a linker containing a disulfide bond." Ex. 2016, 98. The RNA is bound to a column containing avidin, based on the affinity of the biotin for the avidin. *Id.* at 98-99 (Fig. 1). The RNA is "eluted [from the column] by adding dithiothreitol (DTT) to reduce the disulfide bonds linking biotin to the anchor RNA." *Id.* at 98; Ex. 1035 ¶ 31. Relying on testimony by Dr. Romesberg, Illumina states that "Ruby reports that disulfide linkages are cleaved with only ~86% efficiency after more than 100 minutes, which is significantly less than 90% efficient." Paper 61, 12 (citing Ex. 2016, 117-18; Ex. 2009 ¶ 54). The "~86% efficiency" comes from Figure 4 of Ruby, a graph of % RNA eluted using DTT under different conditions. Ex. 2016, 117. Dr. Branchaud did not dispute that Ruby recovered "about 86% of the RNA." Ex. 1035, ¶ 31.

Dr. Romesberg testified that, "[s]ince Ruby's cleavage efficiency under disulfide cleavage conditions is less than 90%, Ruby does not provide an expectation that disulfide cleavage conditions would cleave a 3′-OH protecting group with greater than 90% efficiency." Ex. 2008 ¶ 55.

In response, Dr. Branchaud testified that "even if one of ordinary skill in the art considered the cleavage efficiency of *Ruby* . . . in deciding whether to use such a linker for SBS, one of ordinary skill in the art would know that such cleavage efficiency could be improved by routine experimentation and thus, would not be dissuaded from using such a linker." Ex. 1035 ¶ 37.

Dr. Branchaud's testimony is supported factually. Ruby teaches "[e]lution by reduction of the disulfide bonds on the biotinylated anchor RNA depends on the pH of the buffer, the DTT concentration, and the time of incubation in DTT (Fig. 4) in addition to the type of avidin binding." Ex. 2016, 117-118. Ruby describes manipulating the conditions to alter the elution profile: "By increasing the pH and DTT concentration of the elution buffer slightly, one can effectively elute the RNA during longer incubation times." *Id.* at 118. Thus, Ruby expressly teaches conditions that modify cleavage of the disulfide bond, and that cleavage of the bond can be manipulated by adjusting these conditions. In view of this teaching, one of ordinary skill in the art reasonably would have believed that disulfide bond cleavage could be modified to achieve the desired amount of cleavage.

Dr. Romesberg testified that the 86% value of Ruby could not be exceeded, but did not provide sufficient factual evidence to support this testimony. It is true that Ruby describes an experiment in which, apparently, a maximum of 86% cleavage was obtained, but Ruby did not characterize it as a limit. As Dr. Branchaud testified, it was not critical for Ruby to achieve higher efficiency, so it was not evident why Ruby would have done experimentation to achieve even higher cleavage of the 86% value shown in Figure 4. Ex. 1035 ¶ 37.

Herman

Illumina also cited Herman as evidence that 90% cleavage of a disulfide linkage could not be achieved. Paper 63, 12-13. Herman describes a similar system to Ruby, where RNA is immobilized to a column using a biotin-avidin interaction. Ex. 2017, Abstract, col. 3, ll. 20-25. Herman describes using a biotin attached to a nucleotide base through linker comprising a disulfide bond, as required by the proposed substitute claims. *Id*. at col. 7, ll. 24-34. The disulfide S-S bond is cleaved with a reducing agent, such as DTT or 2-mercaptoethanol. *Id.* at col. 7, l. 47-48; col. 10, ll. 3-19. Herman describes the results of one experiment:

> Bio-SS-DNA with buffer containing 50 mM dithiothreitol resulted in the recovery of a total of 87% of the DNA from the affinity column. Only 7.3% of the 32^{P}-labeled Bio-SS-DNA remained bound to the resin.

Id. at col. 11, ll. 14-17.

Dr. Romesberg makes the same conclusions for Herman that he did for Ruby. That is, since Herman's cleavage was less than 90%, "Herman does not provide an expectation that disulfide cleavage conditions would cleave a 3′-OH protecting group with greater than 90% efficiency." Ex. 2009 ¶ 58. IBS challenged the conclusion that one of ordinary skill in the art reading Herman would have determined the cleavage efficiency to be 87%, providing testimony by Dr. Branchaud that the real efficiency value was above 90% when calculated properly. Ex. 1035 ¶ 36. Dr. Romesberg acknowledged that Herman's values did not "add up," but he testified that there were several possible explanations, of which Dr. Branchaud's was only one. Ex. 2012 (Romesberg Tr.), 193:19–194:13. We are not persuaded,

therefore, that one of ordinary skill in the art would have understood Herman to describe disulfide bond cleavage efficiency above 90%.

The parties cited two additional publications, both co-authored with the same Timothy M. Herman who is listed as inventor of Herman, U.S. Patent No. 4,772, 691: Dawson (1989) (Ex. 1030) (see supra fn. 9), cited by IBS, and Dawson (1991) (Ex. 2039) (see supra fn. 9), cited by Illumina. Dawson (1989) was cited by IBS for its statement in the abstract that "[c]leavage of the disulfide bond in the linker arm of the biotinylated nucleotide resulted in elution of virtually all of the affinity isolated sequences." Ex. 1035 ¶ 37. Dawson (1991) was identified by Illumina for its disclosure that "[r]eduction of the disulfide bond in the biotinylated nucleotide eluted approximately one-half of the affinity isolated chromatin." Ex. 2039, 166.

In other words, the "Herman" publications report varying degrees of cleavage: from "virtually all" in Dawson (1989) (Ex. 1030), to 87% in Herman (Ex. 2017), to approximately 50% in Dawson (1991) (Ex. 2039). It is evident, consistent with Ruby, that the conditions can be routinely varied to achieve a desired level of disulfide bond cleavage.

Moreover, as stated by Dr. Branchaud:

> While it is desirable to achieve a high elution percentage by achieving a high cleavage efficiency of the disulfide linker, unlike SBS, it is not crucial that such elution percentage be greater than 90%. One of ordinary skill in the art would be aware that Ruby and Herman were not necessarily motivated to achieve a high elution percentage and thus a high cleavage efficiency (e.g., greater than 90%). Thus, one of ordinary skill in the art would not be dissuaded from using a disulfide linkage in a modified nucleotide by the cleavage efficiencies shown in Ruby and Herman.

Id. ¶ 35.

In an attempt to rebut this testimony, Illumina cites a 1986 publication by a different group which stated: "Release of the nick-translated probe-plasmid complex from avidin by reduction of the disulfide bond of Bio-19-SS-dUTP gave variable results and was not pursued rigorously." Ex. 2040, 9594. In the ensuing years, however, the Herman group (Exs. 1030, 2017, & 2039) did pursue it and showed that higher cleavage rates could be achieved. Exhibits 1030 & 2017.

Church

Church does not disclose the cleavage efficiency, but shows the results of the experiment in Figure 6. Illumina and IBS dispute the amount of cleavage shown in Figure 6. Because the quality of Figure 6 is so poor, however, the extent of cleavage cannot be determined reliably. A later-filed version of Figure 6 was provided by Illumina to support their claim that cleavage was incomplete, but this Figure was not available until after the August 23, 2002 filing date of the '026 patent. Ex. 2035 (showing that the Figure was not available until October 31, 2002). The later-filed Figure, therefore, does not establish how one of ordinary skill in the art would have interpreted the results of Church at the time the '026 patent was filed. We, therefore, give original filed Figure 6 no weight. Nonetheless, Church suggested disulfide linkers in a sequencing reaction, and carried out an example to show their utility (Ex. 1031, 85-87), providing a reason to have used them in sequencing and a reasonable expectation of success.

Summary

The record contains numerous publications that utilize a disulfide bond linker to join a label to a nucleotide base. Rabani and Church used the linker in the context of DNA sequencing, the primary use for the claimed nucleotides described by Illumina. Ruby, Herman, Dawson (1989), Dawson (1991), Short (referenced in footnote 9), and Rigas, each had used disulfide linkers to attach a label to a base, but not for sequencing purposes. While the prior art reported variability in the disulfide cleavage rates, Illumina has not established by a preponderance of the evidence that efficiency yields above 90% could not be achieved. In particular, Ruby and other publications had no reason to go above whatever cleavage rate was achieved, because it was not critical to their experiments. Herman, in at least one case, described "virtually all" the bond was cleaved. Ex. 1030. Thus, even if 90% efficiency were necessary for a reasonable expectation of success, the ordinary artisan would have expected that such cleavage efficiency of the disulfide bond could be achieved.

Finally, Illumina has not met its burden to show that identical conditions could not be selected in which the disulfide linkage of the cleavable linker is cleavable with less than 90% efficiency and the protecting group is cleavable with greater than 90% efficiency as required by the proposed substitute claims.

H. Objective Evidence of Nonobviousness

Factual considerations that underlie the obviousness inquiry include the scope and content of the prior art, the differences between the prior art and the claimed invention, the level of ordinary skill in the art, and any

relevant secondary considerations. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). Relevant secondary considerations include commercial success, long-felt but unsolved needs, failure of others, and unexpected results. *KSR Int'l Co. v. Teleflex Inc*., 550 U.S. 398, 406 (2007); *In re Soni*, 54 F.3d 746 (Fed. Cir. 1995). Secondary considerations are "not just a cumulative or confirmatory part of the obviousness calculus but constitute independent evidence of nonobviousness . . . [and] enable [] the court to avert the trap of hindsight." *Leo Pharm. Prods., Ltd. v. Rea*, 726 F.3d 1346, 1358 (Fed. Cir. 2013) (internal quotation marks and citations omitted). "This objective evidence must be 'considered as part of all the evidence, not just when the decisionmaker remains in doubt after reviewing the art.'" *Transocean Offshore Deepwater Drilling, Inc. v. Maersk Drilling USA, Inc.*, 699 F.3d 1340, 1349 (Fed. Cir. 2012) (internal citations omitted).

In the Motion to Amend, as objective evidence of nonobviousness, Illumina provided a Declaration by Mr. Eric Vermaas, Illumina's Director of Consumables Product Development, describing sequencing experiments utilizing a nucleotide within the scope of proposed substitute claim 9. Ex. 2028 ¶ 4. The sequencing experiments were performed under Mr. Vermaas's supervision while employed by Illumina. *Id*. at ¶ 5. The sequencing experiments used the four nucleotides dATP, dTTP, dGTP, and dCTP, only one of which – dATP – contained a disulfide base linking a fluorophore to the nucleotide base. *Id*. ¶ 6; Ex. 2009 ¶ 61. Each of these nucleotides also contained an azidomethyl group protecting the 3′-OH. Ex. 2009 ¶ 61.

Mr. Vermaas describes sequential sequencing reactions on PhiX Control DNA for over 150 cycles in which nucleotides were added one at a

time. Ex. 2028 ¶¶ 13-17; Ex. 2009 ¶ 63. After each scan for the fluorophore incorporated, a solution comprising 2 mM tris(hydroxymethyl)-phosphine was added. Ex. 2009 ¶ 64. "The 2 mM tris(hydroxymethyl)phosphine reacts with and cleaves the disulfide linkage of the A [adenine of the dATP] nucleobase," but does not cleave the detectable groups on the other nucleotides. *Id*. According to Dr. Romesberg, "[b]ased on the results presented in the Vermaas declaration, a person of skill in the art would recognize that the yield for cleavage of the disulfide linkage was essentially 100%." *Id*. ¶ 74. Dr. Romesberg concluded:

> Therefore, the disulfide cleavage yield achieved by Illumina was essentially 100%. This is a significant and unexpected improvement over the disulfide cleavages of Ruby (~86%) and Herman (87%). Accordingly, Illumina's proposed claims are nonobvious over the prior art for at least this reason.

Id.

To establish unexpected results, the claimed subject matter must be compared with the closest prior art. *In re Baxter Travenol Labs.*, 952 F.2d 388, 392 (Fed. Cir. 1991). Illumina does not state what references are the closest prior art to the claims. Dr. Romesberg, however, in his declaration, compared the cleavage efficiency reported by Mr. Vermaas with Ruby and Herman. Ex. 2009 ¶ 74.

There are at least two differences between the experiments described in Ruby and Herman, and the experiment described by Mr. Vermaas.

First, while both Ruby and Herman used a nucleotide with a cleavable disulfide bond as did Mr. Vermaas, the references do not use the nucleotide in a sequencing reaction as it has been used in the experiment described by Mr. Vermaas. Ruby involved RNA binding to a column and using the

disulfide linkage to release the bound RNA. Ex. 2016, 98-99. Herman used a system similar to Ruby. Ex. 2017, Abstract, col. 3.

Secondly, the cleavage agents are different. The cleavage agent used in Ruby is DTT (Ex. 2016, 10, "Elution buffer" A and B) and in Herman, the cleavage agent is described as "a reducing agent such as DTT" (Ex. 2017, col. 7, ll. 47-48) with DTT being used in its example (*id*. at col. 10, ll. 47-54, col. 11, ll. 11-22; col. 12, ll. 3-8). Mr. Vermaas describes an experiment that utilized another cleavage agent, 2 mM tris- (hydroxymethyl)phosphine. Ex. 2028 ¶ 10. Illumina did not offer an explanation as to why the phosphine compound was used instead of DTT as used by both Ruby and Herman. Indeed, Church used DTT in a DNA sequencing reaction, similar to the sequencing carried out in the Vermaas experiments, providing an additional reason to have used DTT in Dr. Varmaas's comparison to the prior art. Ex. 1031, Fig. 5; 68, ll. 12-13; 86, ll. 20-23.

There is no testimony that the stated results were due to the claimed nucleotide rather than the reducing agent. Dr. Romesberg testified: "Therefore, the disulfide cleavage yield achieved by Illumina was essentially 100%. This is a significant and unexpected improvement over the disulfide cleavages of Ruby (~86%) and Herman (87%)." Ex. 2009 ¶ 74.

Dr. Romesberg refers to the "disulfide cleavage yield" as being unexpected, but he did not testify that this yield was attributable to the claimed nucleotide configuration, rather than the reducing agent which performs the disulfide bond cleavage.

Illumina has not distinguished the Vermaas results from the prior art by showing that the nucleotide is responsible for the disulfide yield, rather than it being a property of the disulfide bond and the yield being "the mere

recognition" of the bond's "latent properties," which "does not render nonobvious an otherwise known invention." *Baxter*, 952 F.2d at 392; *In re Geisler*, 116 F.3d 1465, 1468 (Fed. Cir. 1997). Absent such evidence, Illumina has not shown that the claimed subject matter possesses "unexpected results relative to the prior art." *Galderma Labs., LP v. Tolmar, Inc*., 737 F3d 731, 738 (Fed. Cir, 2013).

I. Summary

After considering all the evidence as a whole, we conclude that Illumina has not met its burden in showing that the proposed substitute claims are patentable over the prior art considered in this Decision. The Motion to Amend is DENIED to the extent that it requests entry of substitute claims 9-12.

V. MOTIONS TO EXCLUDE EVIDENCE

Both Illumina (Paper 70) and IBS (Paper 67) filed Motions to Exclude Evidence. Illumina's motion is dismissed as moot. IBS's motion is denied in part and dismissed in part as moot.

Illumina's Motion

Illumina requests that Figure 6 from Ex. 1031 (Church), all characterizations of Figure 6 from Ex. 1031, and all arguments based on it be excluded as evidence from this *inter partes* review. Illumina argues that the Figure should be excluded "because it includes poor quality black and white reproductions of images that do not accurately reflect the original underlying data." Paper 70, 1. We agreed with Illumina that the figure of Church is of poor quality and gave it no weight. We, therefore, did not rely

on Figure 6 of Church in reaching our decision. Accordingly, Illumina's motion is dismissed as moot.

IBS's Motion

IBS requests that Illumina's Exhibits 2029, 2030, 2032, 2033, 2035, 2036, and 2039-2042 be excluded as evidence from this *inter partes* review. As we did not rely on Exhibits 2029, 2030, 2032, 2033, and 2042, we dismiss this part of the motion as moot.

Exhibits 2035 and 2036 relate to Figure 6 of Church (Ex. 1031). We did not rely on Figure 6 of Church because it is of such poor quality that the amount disulfide cleavage cannot be determined reliably. To remedy this deficiency, Illumina sought to introduce a substitute Figure 6 from another patent publication by Church. Ex. 2035, 2036. Illumina did not establish that this substitute figure was available prior to the filing date of the '026 patent, however. We, therefore, did not consider it. Consequently, we dismiss this part of the motion as moot.

Exhibits 2039 and 2040 were provided by Illumina as further evidence of the efficacy of disulfide bond cleavage. Paper 64, 3. IBS argues that these Exhibits are not relevant and "without foundation . . . and misleading," because they discuss elution percentages, not cleavage efficiency. Paper 67, 8. We disagree. The Exhibits describe cleavage of a disulfide bond in a nucleotide, the same chemical reaction that is at issue in this *inter partes* review, making them reasonably relevant to the obviousness determination. Nor has IBS explained sufficiently why the documents lack foundation. As to the statement that the Exhibits are "misleading," this argument appears to

go to the weight of the Exhibits, rather than their admissibility. Consequently, we deny IBS's Motion to Exclude Exhibits 2039 and 2040.

VI. ORDER

In consideration of the foregoing, it is

ORDERED that Illumina's Motion to Amend is *denied in part*, to the extent it seeks to add substitute claims 9-12, and *granted in part*, to the extent it seeks to cancel claims 1-8;

FURTHER ORDERED that Illumina's motion to exclude evidence is *dismissed* as moot; and

FURTHER ORDERED that IBS's motion to exclude evidence is *denied in part* with respect to Exhibits 2039 and 2040 and *dismissed in part* as moot with respect to Exhibits 2029, 2030, 2032, 2033, 2035, 2036, and 2042.

IPR2013-00128
Patent 7,057,026 B2

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US007057026B2

(12) **United States Patent**
Barnes et al.

(10) **Patent No.: US 7,057,026 B2**
(45) **Date of Patent: Jun. 6, 2006**

(54) **LABELLED NUCLEOTIDES**

(75) Inventors: **Colin Barnes**, Nr. Saffron Walden (GB); **Shankar Balasubramanian**, Nr. Saffron Walden (GB); **Xiaohai Liu**, Nr. Saffron Walden (GB); **Harold Swerdlow**, Nr. Saffron Walden (GB); **John Milton**, Nr. Saffron Walden (GB)

(73) Assignee: **Solexa Limited**, Nr. Saffron Walden (GB)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 336 days.

(21) Appl. No.: **10/227,131**

(22) Filed: **Aug. 23, 2002**

(65) **Prior Publication Data**

US 2003/0104437 A1 Jun. 5, 2003

(51) **Int. Cl.**

C07H 21/00	(2006.01)
C07H 21/02	(2006.01)
C07H 21/04	(2006.01)
C12Q 1/68	(2006.01)

(52) **U.S. Cl.** **536/23.1**; 435/6; 435/91.1; 435/287.2; 536/25.3; 536/26.6

(58) **Field of Classification Search** 435/6, 435/91.1, 287.2; 536/23.1, 25.3, 26.6
See application file for complete search history.

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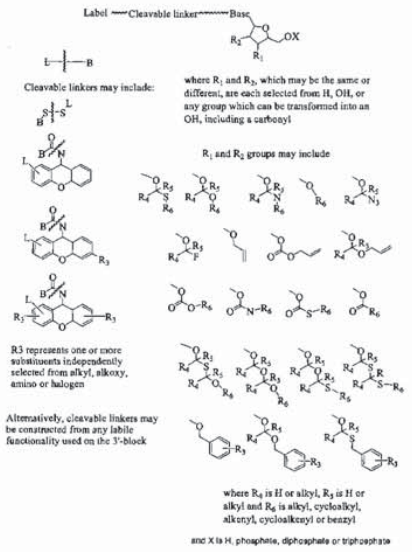
(Continued)

Primary Examiner—Jezia Riley
(74) *Attorney, Agent, or Firm*—Klauber & Jackson LLC

(57) **ABSTRACT**

Nucleosides and nucleotides are disclosed that are linked to detectable labels via a cleavable linker group.

8 Claims, 6 Drawing Sheets



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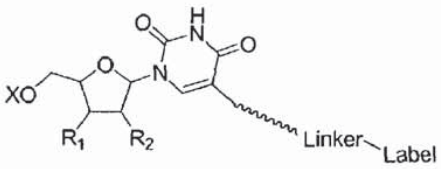
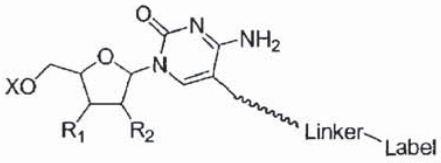
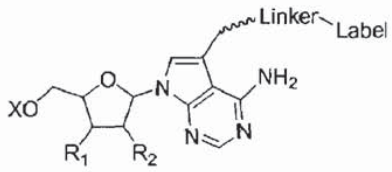


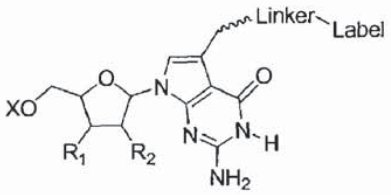
Uridine C5-linker



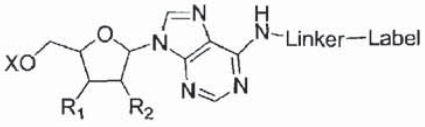
Cytidine C5-linker



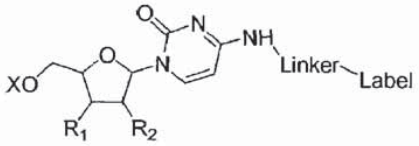
N7 Deazaadenosine C7-linker



N7 Deazaguanosine C7-linker



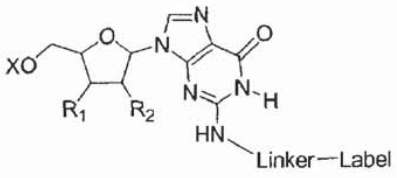
Adenosine N6-linker



Cytidine N4-linker

where R_1 and R_2, which may be the same or different, are each selected from H, OH, or any group which can be transformed into an OH. Suitable groups for R_1 and R_2 are described in Figure 3

X = H, phosphate, diphosphate or triphosphate



Guanosine N2-linker

Fig. 1

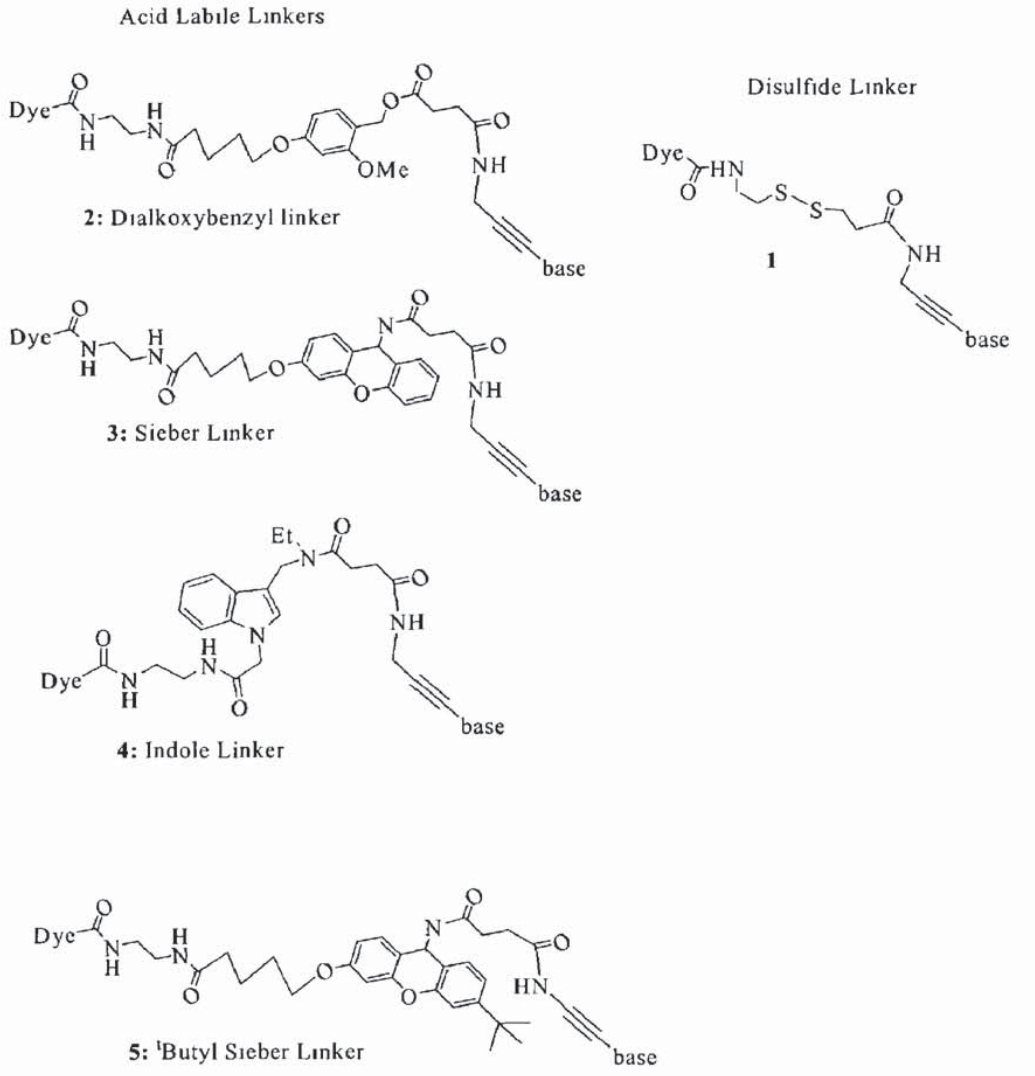
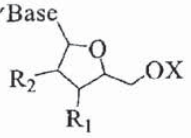
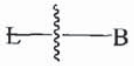

Acid Labile Linkers
Dye
OMe
NH
base
2: Dialkoxybenzyl linker
Disulfide Linker
Dye
HN
S
S
NH
base
1
Dye
3: Sieber Linker
base
Et
Dye
NH
base
4: Indole Linker
Dye
HN
base
5: tButyl Sieber Linker

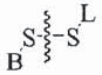
Fig. 2

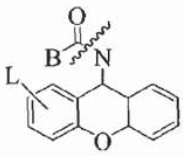
Label ~~~Cleavable linker~~~~~Base

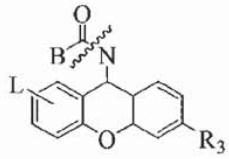


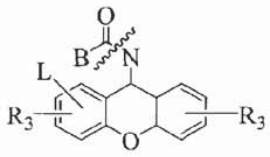


Cleavable linkers may include:







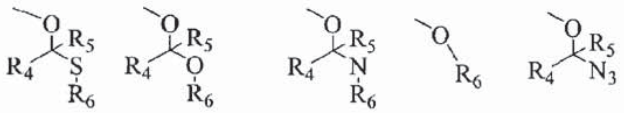


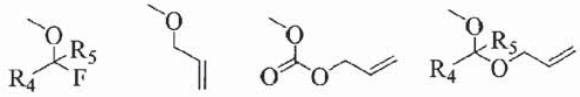
R3 represents one or more substituents independently selected from alkyl, alkoxy, amino or halogen

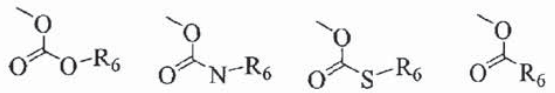
Alternatively, cleavable linkers may be constructed from any labile functionality used on the 3'-block

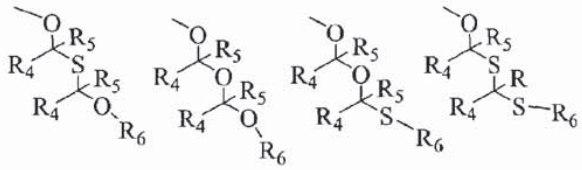
where R_1 and R_2, which may be the same or different, are each selected from H, OH, or any group which can be transformed into an OH, including a carbonyl

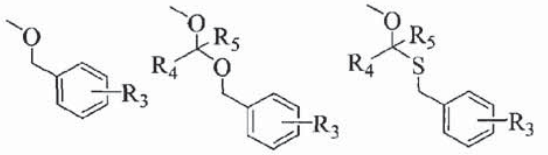
R_1 and R_2 groups may include











where R_4 is H or alkyl, R_5 is H or alkyl and R_6 is alkyl, cycloalkyl, alkenyl, cycloalkenyl or benzyl

and X is H, phosphate, diphosphate or triphosphate

Fig. 3

Klenow exo-
pH7.5
TMR
dUTP
New disulfide cleavable linker (1)
+DTT
0 0.5 1 3 5 0 0.5 1 3 5 min.
Incorporation Cleavage
AG AG
50mM Tris-HCl pH7.5, 10mM NaCl, 0.1mM EDTA
5mM MgCl2, 2uM dNTP-fluor, 100nM SHP 5T hairpin AG oligo,
Klenow exo- (Amersham-Joyce) 10units.
t = 0, 30s, 1,3, 5

Fig. 4

0006

New Acid cleavable Sieber linker (3)
Klenow exo- TMR
pH7.5 dUTP
0 1 3 5 10 min.
AG
50mM Tris-HCl pH7.5, 10mM NaCl, 2mM DTT, 0.1mM EDTA
5mM MgCl2, 2uM dNTP-fluor, 100nM SHP 5T hairpin AG oligo,
Klenow exo- (Amersham-Joyce) 10units.
t = 0, 1, 3, 5, 10

Fig. 5

0007

New Acid cleavable Indole linker (4)
Klenow exo-
pH7.5
TMR
dUTP
0 1 3 5 min
AG
50mM Tris-HCl pH7.5, 10mM NaCl, 2mM DTT, 0.1mM EDTA
5mM MgCl2, 2uM dNTP-fluor, 100nM SHP 5T hairpin AG oligo,
Klenow exo- (Amersham-Joyce) 10units.
t = 0, 1,3, 5

Fig. 6

0008

LABELLED NUCLEOTIDES

RELATED APPLICATION

This application claims benefit of United Kingdom Application No. GB0129012.1, filed Dec. 4, 2001. The entire teachings of the above application are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to labelled nucleotides. In particular, this invention discloses nucleotides having a removable label and their use in polynucleotide sequencing methods.

BACKGROUND

Advances in the study of molecules have been led, in part, by improvement in technologies used to characterise the molecules or their biological reactions. In particular, the study of the nucleic acids DNA and RNA has benefited from developing technologies used for sequence analysis and the study of hybridisation events.

An example of the technologies that have improved the study of nucleic acids, is the development of fabricated arrays of immobilised nucleic acids. These arrays consist typically of a high-density matrix of polynucleotides immobilised onto a solid support material. See, e.g., Fodor et al., *Trends Biotech.* 12:19–26, 1994, which describes ways of assembling the nucleic acids using a chemically sensitized glass surface protected by a mask, but exposed at defined areas to allow attachment of suitably modified nucleotide phosphoramidites. Fabricated arrays can also be manufactured by the technique of "spotting" known polynucleotides onto a solid support at predetermined positions (e.g., Stimpson et al., *Proc. Natl. Acad. Sci. USA* 92:6379–6383, 1995).

A further development in array technology is the attachment of the polynucleotides to the solid support material to form single molecule arrays. Arrays of this type are disclosed in International Patent App. WO 00/06770. The advantage of these arrays is that reactions can be monitored at the single molecule level and information on large numbers of single molecules can be collated from a single reaction.

For DNA arrays to be useful, the sequences of the molecules must be determined. U.S. Pat. No. 5,302,509 discloses a method to sequence polynucleotides immobilised on a solid support. The method relies on the incorporation of 3'-blocked bases A, G, C and T having a different fluorescent label to the immobilised polynucleotide, in the presence of DNA polymerase. The polymerase incorporates a base complementary to the target polynucleotide, but is prevented from further addition by the 3'-blocking group. The label of the incorporated base can then be determined and the blocking group removed by chemical cleavage to allow further polymerisation to occur.

Welch et al. (*Chem. Eur. J.* 5(3):951–960, 1999) describes the synthesis of nucleotide triphosphates modified with a 3'-O-blocking group that is photolabile and fluorescent. The modified nucleotides are intended for use in DNA sequencing experiments. However, these nucleotides proved to be difficult to incorporate onto an existing polynucleotide, due to an inability to fit into the polymerase enzyme active site.

Zhu et al. (*Cytometry* 28:206–211, 1997) also discloses the use of fluorescent labels attached to a nucleotide via the base group. The labelled nucleotides are intended for use in fluorescence in situ hybridisation (FISH) experiments, where a series of incorporated labelled nucleotides is required to produce a fluorescent "bar code".

SUMMARY OF THE INVENTION

In the present invention, a nucleoside or nucleotide molecule is linked to a detectable label via a cleavable linker group attached to the base, rendering the molecule useful in techniques using Labelled nucleosides or nucleotides, e.g., sequencing reactions, polynucleotide synthesis, nucleic acid amplification, nucleic acid hybridization assays, single nucleotide polymorphism studies, and other techniques using enzymes such as polymerases, reverse transcriptases, terminal transferases, or other DNA modifying enzymes. The invention is especially useful in techniques that use Labelled dNTPs, such as nick translation, random primer labeling, end-labeling (e.g., with terminal deoxynucleotidyltransferase), reverse transcription, or nucleic acid amplification. The molecules of the present invention are in contrast to the prior art, where the label is attached to the ribose or deoxyribose sugar, or where the label is attached via a non-cleavable linker.

According to a first aspect of the invention, a nucleotide or nucleoside molecule, or an analog thereof, has a base that is linked to a detectable label via a cleavable linker.

The invention features a nucleotide or nucleoside molecule, having a base that is linked to a detectable label via a cleavable linker. The base can be a purine, or a pyrimidine. The base can be a deazapurine. The molecule can have a ribose or deoxyribose sugar moiety. The ribose or deoxyribose sugar can include a protecting group attached via the 2' or 3' oxygen atom. The protecting group can be removed to expose a 3'-OH. The molecule can be a deoxyribonucleotide triphosphate. The detectable label can be a fluorophore. The linker can be an acid labile linker, a photolabile linker, or can contain a disulphide linkage.

The invention also features a method of labeling a nucleic acid molecule, where the method includes incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, where the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker. The incorporating step can be accomplished via a terminal transferase, a polymerase or a reverse transcriptase. The base can be a purine, or a pyrimidine. The base can be a deazapurine. The nucleotide or nucleoside molecule can have a ribose or deoxyribose sugar moiety. The ribose or deoxyribose sugar can include a protecting group attached via the 2' or 3' oxygen atom. The protecting group can be removed to expose a 3'-OH group. The molecule can be a deoxyribonucleotide triphosphate. The detectable label can be a fluorophore. The linker can be an acid labile linker, a photolabile linker, or can contain a disulphide linkage. The detectable label and/or the cleavable linker can be of a size sufficient to prevent the incorporation of a second nucleotide or nucleoside into the nucleic acid molecule.

In another aspect, the invention features a method for determining the sequence of a target single-stranded polynucleotide, where the method includes monitoring the sequential incorporation of complementary nucleotides, where the nucleotides each have a base that is linked to a detectable label via a cleavable linker, and where the identity of each nucleotide incorporated is determined by detection of the label linked to the base, and subsequent removal of the label.

The invention also features a method for determining the sequence of a target single-stranded polynucleotide, where the method includes: (a) providing nucleotides, where the

nucleotides have a base that is linked to a detectable label via a cleavable linker, and where the detectable label linked to each type of nucleotide can be distinguished upon detection from the detectable label used for other types of nucleotides; (b) incorporating a nucleotide into the complement of the target single stranded polynucleotide; (c) detecting the label of the nucleotide of (b), thereby determining the type of nucleotide incorporated; (d) removing the label of the nucleotide of (b); and (e) optionally repeating steps (b)–(d) one or more times; thereby determining the sequence of a target single-stranded polynucleotide.

In the methods described herein, each of the nucleotides can be brought into contact with thew target sequentially, with removal of non-incorporated nucleotides prior to addition of the next nucleotide, where detection and removal of the label is carried out either after addition of each nucleotide, or after addition of all four nucleotides.

In the methods, all of the nucleotides can be brought into contact with the target simultaneously, i.e., a composition comprising all of the different nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label(s).

The methods can comprise a first step and a second step, where in the first step, a first composition comprising two of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where in the second step, a second composition comprising the two nucleotides not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where the first steps and the second step can be optionally repeated one or more times.

The methods described herein can also comprise a first step and a second step, where in the first step, a composition comprising one of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where in the second step, a second composition comprising the three nucleotides not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where the first steps and the second step can be optionally repeated one or more times.

The methods described herein can also comprise a first step and a second step, where in the first step, a first composition comprising three of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where in the second step, a composition comprising the nucleotide not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where the first steps and the second step can be optionally repeated one or more times.

In a further aspect, the invention features a kit, where the kit includes: (a) individual the nucleotides, where each nucleotide has a base that is linked to a detectable label via a cleavable linker, and where the detectable label linked to each nucleotide can be distinguished upon detection from the detectable label used for other three nucleotides; and (b) packaging materials therefor. The kit can further include an enzyme and buffers appropriate for the action of the enzyme.

The nucleotides/nucleosides are suitable for use in many different DNA-based methodologies, including DNA synthesis and DNA sequencing protocols.

According to another aspect of the invention, a method for determining the sequence of a target polynucleotide comprises monitoring the sequential incorporation of complementary nucleotides, wherein the nucleotides comprise a detectable label linked to the base portion of the nucleotide via a cleavable linker, incorporation is detected by monitoring the label, and the label is removed to permit further nucleotide incorporation to occur.

DECRIPTION OF THE DRAWINGS

FIG. **1** shows examplary nucleotide structures useful in the invention. For each structure, X can be H, phosphate, diphosphate or triphosphate. R_1 and R_2 can be the same or different, and can be selected from H, OH, or any group which can be transformed into an OH, including, but not limited to, a carbonyl. Some suitable functional groups for R_1 and R_2 include the structures shown in FIG. **3**.

FIG. **2** shows structures of linkers useful in the invention, including (1) disulfide linkers and acid labile linkers, (2) dialkoxybenzyl linkers, (3) Sieber linkers, (4) indole linkers and (5) t-butyl Sieber linkers.

FIG. **3** shows some functional molecules useful in the invention, including some cleavable linkers and some suitable hydroxyl protecting groups. In these structures, R_1 and R_2 may be the same of different, and can be H, OH, or any group which can be transformed into an OH group, including a carbonyl. R_3 represents one or more substituents independently selected from alkyl, alkoxyl, amino or halogen groups. Alternatively, cleavable linkers may be constructed from any labile functionality used on the 3'-block. R_4 and R_5 can be H or alkyl, and R_6 can be alkyl, cycloalkyl, alkenyl, cycloalkenyl or benzyl. X can be H, phosphate, diphosphate or triphosphate.

FIG. **4** shows a denaturing gel showing the incorporation of the triphosphate of Example 1 using Klenow polymerase.

FIG. **5** shows a denaturing gel showing the incorporation of the triphosphate of Example 3 using Klenow polymerase.

FIG. **6** shows a denaturing gel showing the incorporation of the triphosphate of Example 4 using Klenow polymerase.

DETAILED DESCRIPTION

The present invention relates to nucleotides and nucleosides that are modified by attachment of a label via a cleavable linker, thereby rendering the molecule useful in techniques where the labelled molecule is to interact with an enzyme, such as sequencing reactions, polynucleotide synthesis, nucleic acid amplification, nucleic acid hybridization assays, single nucleotide polymorphism studies, techniques using enzymes such as polymerase, reverse transcriptase, terminal transferase, techniques that use Labelled dNTPs (e.g., nick translation, random primer labeling, end-labeling (e.g., with terminal deoxynucleotidyltransferase), reverse transcription, or nucleic acid amplification).

As is known in the art, a "nucleotide" consists of a nitrogenous base, a sugar, and one or more phosphate groups. In RNA, the sugar is a ribose, and in DNA is a deoxyribose, i.e., a sugar lacking a hydroxyl group that is present in ribose. The nitrogenous base is a derivative of purine or pyrimidine. The purines are adenosine (A) and guanidine (G), and the pyrimidines are cytidine (C) and thymidine (T) (or in the context of RNA, uracil (U)). The C-1 atom of deoxyribose is bonded to N-1 of a pyrimidine

or N-9 of a purine. A nucleotide is also a phosphate ester of a nucleoside, with esterification occurring on the hydroxyl group attached to C-5 of the sugar. Nucleotides are usually mono, di- or triphosphates.

A "nucleoside" is structurally similar to a nucleotide, but are missing the phosphate moieties. An example of a nucleoside analog would be one in which the label is linked to the base and there is no phosphate group attached to the sugar molecule.

Although the base is usually referred to as a purine or pyrimidine, the skilled person will appreciate that derivatives and analogs are available which do not alter the capability of the nucleotide or nucleoside to undergo Watson-Crick base pairing. "Derivative" or "analog" means a compound or molecule whose core structure is the same as, or closely resembles that of, a parent compound, but which has a chemical or physical modification, such as a different or additional side groups, which allows the derivative nucleotide or nucleoside to be linked to another molecule. For example, the base can be a deazapurine. The derivatives should be capable of undergoing Watson-Crick pairing. "Deivative" and "analog" also mean a synthetic nucleotide or nucleoside derivative having modified base moieties and/or modified sugar moieties. Such derivatives and analogs are discussed in, e.g., Scheit, *Nucleotide Analogs* (John Wiley & Son, 1980) and Uhlman et al., *Chemical Reviews* 90:543–584, 1990. Nucleotide analogs can also comprise modified phosphodiester linkages, including phosphorothioate, phosphorodithioate, alkylphosphonate, phosphoranilidate and phosphoramidate linkages. The analogs should be capable of undergoing Watson-Crick base pairing. "Derivative" and "analog", as used herein, may be used interchangeably, and are encompassed by the terms "nucleotide" and "nucleoside" as defined herein.

The present invention can make use of conventional detectable labels. Detection can be carried out by any suitable method, including fluorescence spectroscopy or by other optical means. The preferred label is a fluorophore, which, after absorption of energy, emits radiation at a defined wavelength. Many suitable fluorescent labels are known. For example, Welch et al. (*Chem. Eur. J.* 5(3): 951–960, 1999) discloses dansyl-functionalised fluorescent moieties that can be used in the present invention. Zhu et al. (*Cytometry* 28:206–211, 1997) describes the use of the fluorescent labels Cy3 and Cy5, which can also be used in the present invention. Labels suitable for use are also disclosed in Prober et al. (*Science* 238:336–341, 1987); Connell et al. (*BioTechniques* 5(4):342–384, 1987), Ansorge et al. (*Nucl. Acids Res.* 15(11):4593–4602, 1987) and Smith et al. (*Nature* 321:674, 1986). Other commercially available fluorescent labels include, but are not limited to, fluorescein, rhodamine (including TMR, texas red and Rox), alexa, bodipy, acridine, coumarin, pyrene, benzanthracene and the cyanins.

Multiple labels can also be used in the invention. For example, bi-fluorophore FRET cassettes (*Tet. Letts.* 46:8867–8871, 2000) are well known in the art and can be utilised in the present invention. Multi-fluor dendrimeric systems (*J. Amer. Chem. Soc.* 123:8101–8108, 2001) can also be used.

Although fluorescent labels are preferred, other forms of detectable labels will be apparent as useful to those of ordinary skill. For example, microparticles, including quantum dots (Empodocles, et al., *Nature* 399:126–130, 1999), gold nanoparticles (Reichert et al., *Anal. Chem.* 72:6025–6029, 2000), microbeads (Lacoste et al., *Proc. Natl. Acad. Sci USA* 97(17):9461–9466, 2000), and tags detectable by mass spectrometry can all be used.

Multi-component labels can also be used in the invention. A multi-component label is one which is dependent on the interaction with a further compound for detection. The most common multi-component label used in biology is the biotin-streptavidin system. Biotin is used as the label attached to the nucleotide base. Streptavidin is then added separately to enable detection to occur. Other multi-component systems are available. For example, dinitrophenol has a commercially available fluorescent antibody that can be used for detection.

The label (or label and linker construct) can be of a size or structure sufficient to act as a block to the incorporation of a further nucleotide onto the nucleotide of the invention. This permits controlled polymerization to be carried out. The block can be due to steric hindrance, or can be due to a combination of size, charge and structure.

The invention will be further described with reference to nucleotides. However, unless indicated otherwise, the reference to nucleotides is also intended to be applicable to nucleosides. The invention will also be further described with reference to DNA, although the description will also be applicable to RNA, PNA, and other nucleic acids, unless otherwise indicated.

The modified nucleotides of the invention use a cleavable linker to attach the label to the nucleotide. The use of a cleavable linker ensures that the label can, if required, be removed after detection, avoiding any interfering signal with any labelled nucleotide incorporated subsequently.

Cleavable linkers are known in the art, and conventional chemistry can be applied to attach a linker to a nucleotide base and a label. The linker can be cleaved by any suitable method, including exposure to acids, bases, nucleophiles, electrophiles, radicals, metals, reducing or oxidising agents, light, temperature, enzymes etc. Suitable linkers can be adapted from standard chemical blocking groups, as disclosed in Greene & Wuts, *Protective Groups in Organic Synthesis,* John Wiley & Sons. Further suitable cleavable linkers used in solid-phase synthesis are disclosed in Guillier et al. (*Chem. Rev.* 100:2092–2157, 2000).

The use of the term "cleavable linker" is not meant to imply that the whole linker is required to be removed from the nucleotide base. The cleavage site can be located at a position on the linker that ensures that part of the linker remains attached to the nucleotide base after cleavage.

The linker can be attached at any position on the nucleotide base provided that Watson-Crick base pairing can still be carried out. In the context of purine bases, it is preferred if the linker is attached via the 7 position of the purine or the preferred deazapurine analogue, via an 8-modified purine, via an N-6 modified adenosine or an N-2 modified guanine. For pyrimidines, attachment is preferably via the 5 position on cytidine, thymidine or uracil and the N-4 position on cytosine. Suitable nucleotide structures are shown in FIG. **1**. For each structure in FIG. **1**, X can be H, phosphate, diphosphate or triphosphate. R_1 and R_2 can be the same or different, and can be selected from H, OH, or any group which can be transformed into an OH, including, but not limited to, a carbonyl. Some suitable functional groups for R_1 and R_2 include the structures shown in FIG. **3**.

Suitable linkers are shown in FIG. **2** and include, but are not limited to, disulfide linkers (1), acid labile linkers (2, 3, 4 and 5; including dialkoxybenzyl linkers (e.g., 2), Sieber linkers (e.g., 3), indole linkers (e.g., 4), t-butyl Sieber linkers (e.g., 5)), electrophilically cleavable linkers, nucleophilically cleavable linkers, photocleavable linkers, cleavage

under reductive conditions, oxidative conditions, cleavage via use of safety-catch linkers, and cleavage by elimination mechanisms.

A. Electrophilically Cleaved Linkers.

Electrophilically cleaved linkers are typically cleaved by protons and include cleavages sensitive to acids. Suitable linkers include the modified benzylic systems such as trityl, p-alkoxybenzyl esters and p-alkoxybenzyl amides. Other suitable linkers include tert-butyloxycarbonyl (Boc) groups and the acetal system (e.g., as is shown in FIG. **3** as $O—C(R_4)(R_5)—O—R_6$.

The use of thiophilic metals, such as nickel, silver or mercury, in the cleavage of thioacetal or other sulphur-containing protecting groups can also be considered for the preparation of suitable linker molecules.

B. Nucleophilically Cleaved Linkers.

Nucleophilic cleavage is also a well recognised method in the preparation of linker molecules. Groups such as esters that are labile in water (i.e., can be cleaved simply at basic pH) and groups that are labile to non-aqueous nucleophiles, can be used. Fluoride ions can be used to cleave silicon-oxygen bonds in groups such as triisopropyl silane (TIPS) or t-butyldimethyl silane (TBDMS).

C. Photocleavable Linkers.

Photocleavable linkers have been used widely in carbohydrate chemistry. It is preferable that the light required to activate cleavage does not affect the other components of the modified nucleotides. For example, if a fluorophore is used as the label, it is preferable if this absorbs light of a different wavelength to that required to cleave the linker molecule. Suitable linkers include those based on O-nitrobenyl compounds and nitroveratryl compounds. Linkers based on benzoin chemistry can also be used (Lee et al., *J. Org. Chem.* 64:3454–3460, 1999).

D. Cleavage Under Reductive Conditions

There are many linkers known that are susceptible to reductive cleavage. Catalytic hydrogenation using palladium-based catalysts has been used to cleave benzyl and benzyloxycarbonyl groups. Disulphide bond reduction is also known in the art.

E. Cleavage Under Oxidative Conditions

Oxidation-based approaches are well known in the art. These include oxidation of p-alkoxybenzyl groups and the oxidation of sulphur and selenium linkers. The use of aqueous iodine to cleave disulphides and other sulphur or selenium-based linkers is also within the scope of the invention.

F. Safety-catch Linkers

Safety-catch linkers are those that cleave in two steps. In a preferred system the first step is the generation of a reactive nucleophilic center followed by a second step involving an intra-molecular cyclization that results in cleavage. For example, levulinic ester linkages can be treated with hydrazine or photochemistry to release an active amine, which can then be cyclised to cleave an ester elsewhere in the molecule (Burgess et al., *J. Org. Chem.* 62:5165–5168, 1997).

G. Cleavage by Elimination Mechanisms

Elimination reactions can also be used. For example, the base-catalysed elimination of groups such as Fmoc and cyanoethyl, and palladium-catalysed reductive elimination of allylic systems, can be used.

As well as the cleavage site, the linker can comprise a spacer unit. The spacer distances the nucleotide base from the cleavage site or label. The length of the linker is unimportant provided that the label is held a sufficient distance from the nucleotide so as not to interfere with any interaction between the nucleotide and an enzyme.

The modified nucleotides can also comprise additional groups or modifications to the sugar group. For example, a dideoxyribose derivative, lacking two oxygens on the ribose ring structure (at the 2' and 3' positions), can be prepared and used as a block to further nucleotide incorporation on a growing oligonucleotide strand. The ribose ring can also be modified to include a protecting group at the 3' position or a group that can be transformed or modified to form a 3' OH group. The protecting group is intended to prevent nucleotide incorporation onto a nascent polynucleotide strand, and can be removed under defined conditions to allow polymerisation to occur. In contrast to the prior art, there is no detectable label attached at the ribose 3' position. This ensures that steric hindrance with the polymerase enzyme is reduced, while still allowing control of incorporation using the protecting group.

The skilled person will appreciate how to attach a suitable protecting group to the ribose ring to block interactions with the 3'-OH. The protecting group can be attached directly at the 3' position, or can be attached at the 2' position (the protecting group being of sufficient size or charge to block interactions at the 3' position). Alternatively, the protecting group can be attached at both the 3' and 2' positions, and can be cleaved to expose the 3'OH group.

Suitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in Green and Wuts, supra. Some examples of such protecting groups are shown in FIG. **3**. The protecting group should be removable (or modifiable) to produce a 3' OH group. The process used to obtain the 3' OH group can be any suitable chemical or enzymic reaction.

The labile linker may consist of functionality cleavable under identical conditions to the block. This will make the deprotection process more efficient as only a single treatment will be required to cleave both the label and the block. Thus the linker may contain functional groups as described in FIG. **3**, which could be cleaved with the hydroxyl functionality on either the residual nucleoside or the removed label. The linker may also consist of entirely different chemical functionality that happens to be labile to the conditions used to cleave the block.

The term "alkyl" covers both straight chain and branched chain alkyl groups. Unless the context indicates otherwise, the term "alkyl" refers to groups having 1 to 8 carbon atoms, and typically from 1 to 6 carbon atoms, for example from 1 to 4 carbon atoms. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-pentyl, 2-pentyl, 3-pentyl, 2-methyl butyl, 3-methyl butyl, and n-hexyl and its isomers.

Examples of cycloalkyl groups are those having from 3 to 10 ring atoms, particular examples including those derived from cyclopropane, cyclobutane, cyclopentane, cyclohexane and cycloheptane, bicycloheptane and decalin.

Examples of alkenyl groups include, but are not limited to, ethenyl (vinyl), 1-propenyl, 2-propenyl (allyl), isopropenyl, butenyl, buta-1,4-dienyl, pentenyl, and hexenyl.

Examples of cycloalkenyl groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl and cyclohexenyl.

The term alkoxy refers to C_{1-6} alkoxy unless otherwise indicated: —OR, wherein R is a C_{1-6} alkyl group. Examples of C_{1-6} alkoxy groups include, but are not limited to, —OMe (methoxy), —OEt (ethoxy), —O(nPr) (n-propoxy),

—O(iPr) (isopropoxy), —O(nBu) (n-butoxy), —O(sBU) (sec-butoxy), —O(iBu) (isobutoxy), and —O(tBu) (tert-butoxy).

The term amino refers to groups of type NR^1R^2, wherein R^1 and R^2 are independently selected from hydrogen, a C_{1-6} alkyl group (also referred to as C_{1-6} alkylamino or di-C_{1-6} alkylamino).

The term "halogen" as used herein includes fluorine, chlorine, bromine and iodine.

The nucleotide molecules of the present invention are suitable for use in many different methods where the detection of nucleotides is required.

DNA sequencing methods, such as those outlined in U.S. Pat. No. 5,302,509 can be carried out using the nucleotides.

A method for determining the sequence of a target polynucleotide can be carried out by contacting the target polynucleotide separately with the different nucleotides to form the complement to that of the target polynucleotide, and detecting the incorporation of the nucleotides. Such a method makes use of polymerisation, whereby a polymerase enzyme extends the complementary strand by incorporating the correct nucleotide complementary to that on the target. The polymerisation reaction also requires a specific primer to initiate polymerisation.

For each cycle, the incorporation of the labelled nucleotide is carried out by the polymerase enzyme, and the incorporation event is then determined. Many different polymerase enzymes exist, and it will be evident to the person of ordinary skill which is most appropriate to use. Preferred enzymes include DNA polymerase I, the Klenow fragment, DNA polymerase III, T4 or T7 DNA polymerase, Taq polymerase or vent polymerase. A polymerase engineered to have specific properties can also be used.

The sequencing methods are preferably carried out with the target polynucleotide arrayed on a solid support. Multiple target polynucleotides can be immobilised on the solid support through linker molecules, or can be attached to particles, e.g., microspheres, which can also be attached to a solid support material.

The polynucleotides can be attached to the solid support by a number of means, including the use of biotin-avidin interactions. Methods for immobilizing polynucleotides on a solid support are well known in the art, and include lithographic techniques and "spotting" individual polynucleotides in defined positions on a solid support. Suitable solid supports are known in the art, and include glass slides and beads, ceramic and silicon surfaces and plastic materials. The support is usually a flat surface although microscopic beads (microspheres) can also be used and can in turn be attached to another solid support by known means. The microspheres can be of any suitable size, typically in the range of from 10 nm to 100 nm in diameter. In a preferred embodiment, the polynucleotides are attached directly onto a planar surface, preferably a planar glass surface. Attachment will preferably be by means of a covalent linkage. Preferably, the arrays that are used are single molecule arrays that comprise polynucleotides in distinct optically resolvable areas, e.g., as disclosed in International App. No. WO 00/06770.

The sequencing method can be carried out on both single polynucleotide molecule and multi-polynucleotide molecule arrays, i.e., arrays of distinct individual polynucleotide molecules and arrays of distinct regions comprising multiple copies of one individual polynucleotide molecule. Single molecule arrays allow each individual polynucleotide to be resolved separately. The use of single molecule arrays is preferred. Sequencing single molecule arrays non-destructively allows a spatially addressable array to be formed.

The method makes use of the polymerisation reaction to generate the complementary sequence of the target. The conditions necessary for polymerisation to occur will be apparent to the skilled person.

To carry out the polymerase reaction it will usually be necessary to first anneal a primer sequence to the target polynucleotide, the primer sequence being recognised by the polymerase enzyme and acting as an initiation site for the subsequent extension of the complementary strand. The primer sequence may be added as a separate component with respect to the target polynucleotide. Alternatively, the primer and the target polynucleotide may each be part of one single stranded molecule, with the primer portion forming an intramolecular duplex with a part of the target, i.e., a hairpin loop structure. This structure may be immobilised to the solid support at any point on the molecule. Other conditions necessary for carrying out the polymerase reaction, including temperature, pH, buffer compositions etc., will be apparent to those skilled in the art.

The modified nucleotides of the invention are then brought into contact with the target polynucleotide, to allow polymerisation to occur. The nucleotides may be added sequentially, i.e., separate addition of each nucleotide type (A, T, G or C), or added together. If they are added together, it is preferable for each nucleotide type to be labelled with a different label.

This polymerisation step is allowed to proceed for a time sufficient to allow incorporation of a nucleotide.

Nucleotides that are not incorporated are then removed, for example, by subjecting the array to a washing step, and detection of the incorporated labels may then be carried out.

Detection may be by conventional means, for example if the label is a fluorescent moiety, detection of an incorporated base may be carried out by using a confocal scanning microscope to scan the surface of the array with a laser, to image a fluorophore bound directly to the incorporated base. Alternatively, a sensitive 2-D detector, such as a charge-coupled detector (CCD), can be used to visualise the individual signals generated. However, other techniques such as scanning near-field optical microscopy (SNOM) are available and may be used when imaging dense arrays. For example, using SNOM, individual polynucleotides may be distinguished when separated by a distance of less than 100 nm, e.g., 10 nm to 10 μm. For a description of scanning near-field optical microscopy, see Moyer et al., *Laser Focus World* 29:10, 1993. Suitable apparatus used for imaging polynucleotide arrays are known and the technical set-up will be apparent to the skilled person.

After detection, the label may be removed using suitable conditions that cleave the linker.

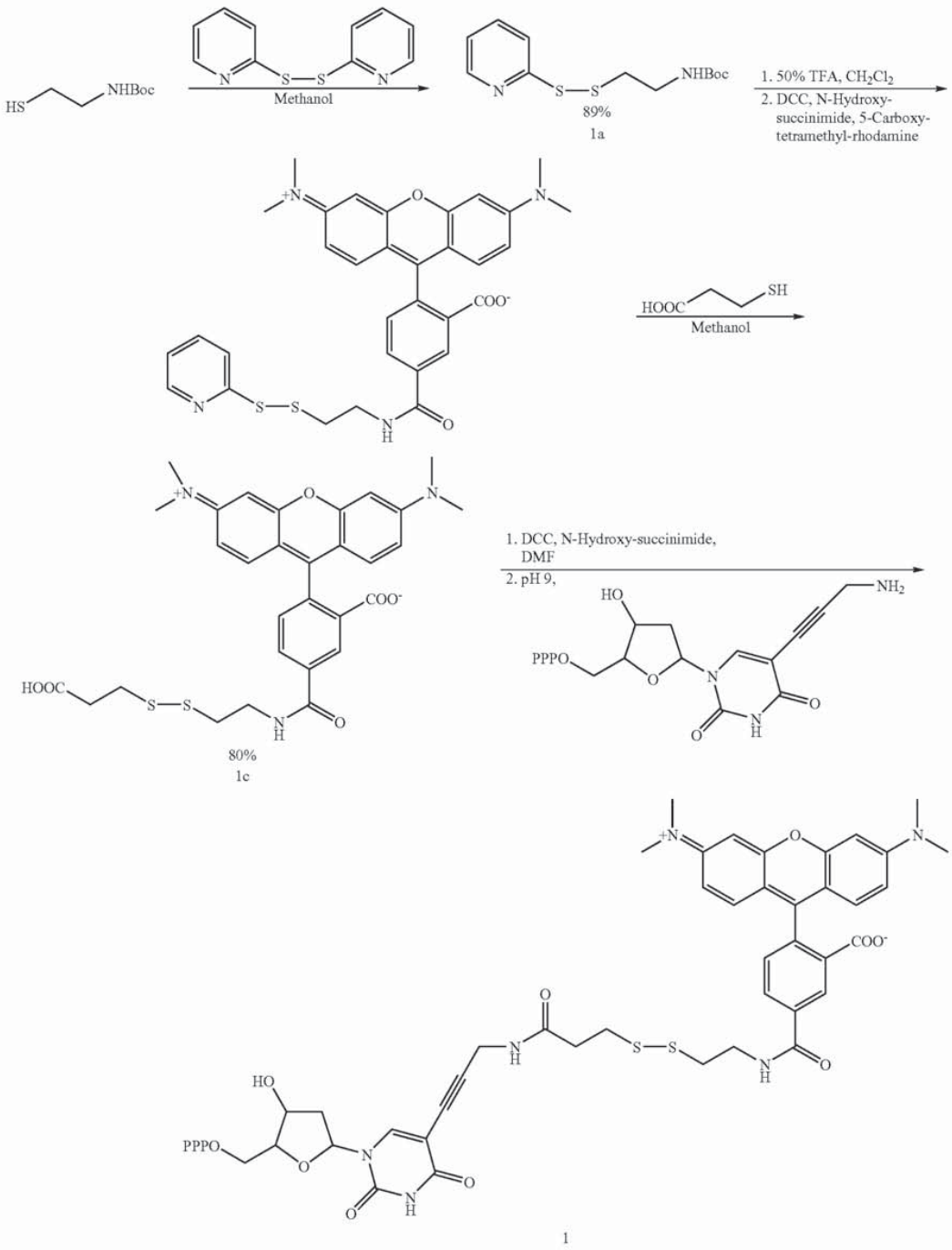
The use of the modified nucleotides is not limited to DNA sequencing techniques, and other techniques, including polynucleotide synthesis, DNA hybridisation assays and single nucleotide polymorphism studies, may also be carried out using nucleotides of the invention. Any technique that involves the interaction between a nucleotide and an enzyme may make use of the molecules of the invention. For example, the molecule may be used as a substrate for a reverse transcriptase or terminal transferase enzyme.

Suitable structures are described in the following Examples and are shown in the accompanying drawings.

EXAMPLES

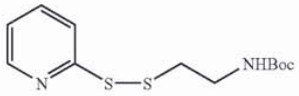
Example 1

Synthesis of Disulfide Linker



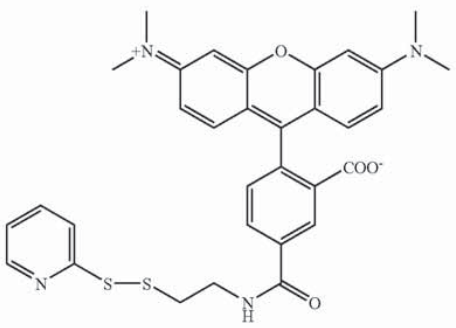
-continued

1a



tButyl-N-(2-mercaptoethyl) carbamate (3 mmol, 0.5 mL) was added dropwise to a solution of 1.32 g (6.0 mmol) aldrithiol in 15 mL MeOH. After 1.5 h the reaction had gone to completion and the solvent was evaporated. The crude product was purified by chromatography on silica with ethyl acetate:petroleum ether (1:4). Product 1*a* was obtained as a slightly yellow oil (0.76 g, 2.67 mmol, 89%). ^{1}H NMR (500 Mhz, D_6-DMSO): δ=1.38 (s, 9 H, tBu), 2.88 (t, J=6.6 Hz, 2 H, SCH_2)3.20(q, J=6.6 Hz, 2 H, CH_2NH), 7.02 (bs, 1 H, NH), 7.24 (ddd, J=7.3 Hz, J=4.9 Hz, J=1.0 Hz, 1 H, H-5), 7.77 (dt, J=8.1 Hz, J=1.0 Hz, 1 H, H-3), 7.82 (ddd, J=8.1 Hz, J=7.4 Hz, J=1.8 Hz, 1 H, H-4), 8.46 (ddd, J=4.9 Hz, J=1.8 Hz, J=1.0 Hz, 1 H, H-6).

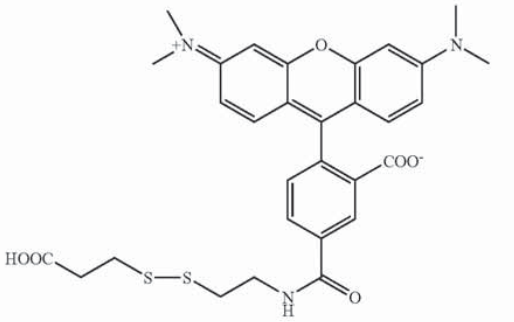
1b



To deprotect the amine of 1*a*, 17 mg of 1*a* (60 μmol) was dissolved in a mixture of 0.5 mL DCM and 0.5 mL trifluoracetic acid. This mixture was stirred for 2.5 h at rt and then the solvents were removed under reduced pressure. The residue was three times redissolved in 2 mL DCM and evaporated to dryness. The deprotected product was dried under high vacuum for 3 h and then dissolved in 1 mL dry DMF. It was assumed that the deprotection had gone to completion.

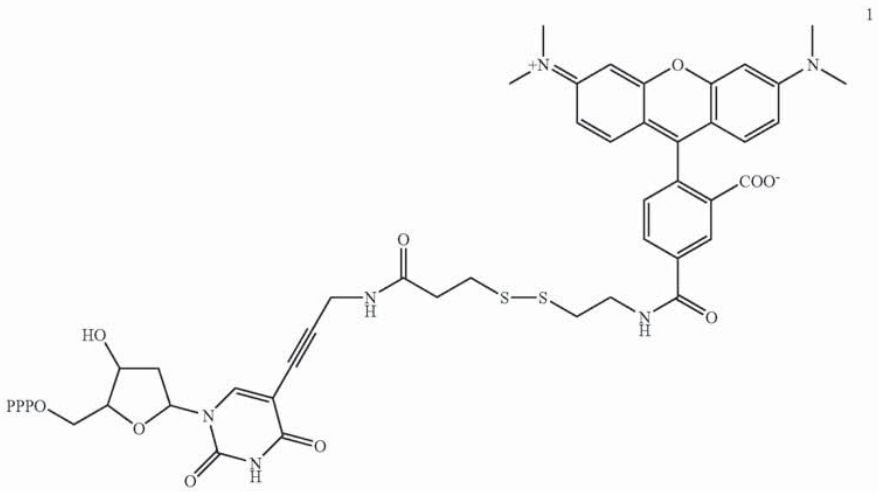
To a solution of 15 mg 5-carboxy tetra methyl rhodamine (35 μmol) in 2 mL DMF were added 8.0 mg N-hydroxy succinimide (70 μmol) and 7.8 mg DCC (38 μmol). The mixture was stirred for 6 h in the dark. Then 22 μl DIPEA (126 μmol) and the solution of deprotected 1*a* in 1 mL DMF were added. After stirring the reaction mixture overnight in the dark, the solvent was removed under reduced pressure. The residue was dissolved in DCM and washed with saturated NaCl solution. After drying over $MgSO_4$ the crude mixture was purified on silica with $CHCl_3$:MeOH (3:1) as solvent. 1*b* was isolated as a dark red solid in 90% yield (19.2 mg, 31.4 μmol). ^{1}H NMR (500 MHz, D_6-DMSO): δ=3.09 (t, J=6.7 Hz, 2 H, SCH_2), 3.63 (q, J=6.2 Hz, 2 H, CH_2NH), 6.48–6.53 (m, 6 H, H-Anthracene), 7.23–7.26 [m, 1 H, H-5 (pyridine)], 7.32 (d, J=7.9 Hz, 1 Hz, H-3), 7.81–7.82 [m, 2 H, H-3+H-4 (pyridine)], 8.21 (d, J=7.9 Hz, 1 H, H-4), 8.43 (s, 1 H, H-6), 8.47 [dt, J=4.7 Hz, J=1.3 Hz, 1 H, H-6 (pyridine)], 9.03 (t, J=5.2 Hz, 1 H, NH).

1c



Mercaptopropionic acid (20.6 μmol, 1.8 μl) was added to a solution of 19.6 mg 1*b* (32.7 μmol) in 2 mL MeOH. The mixture was stirred for 2.5 h in the dark. The solvent was removed under reduced pressure. The crude product was purified by chromatography on silica with $CHCl_3$:MeOH:AcOH 15:1:0.5 as the solvent mixture. 15.5 mg (26 μmol, 80%) dark red crystals 1*c* could be isolated. ^{1}H NMR (500 MHz, D_2O): δ=2.53 (t, J=7.0 Hz, 2 H, CH_2COOH), 2.88 (t, J=7.0 Hz, 2 H, CH_2CH_2COOH), 2.96–2.99 (m, 2 H, CH_2CH_2NH), 3.73 (t, J=6.3 Hz, 2 H, CH_2NH), 6.53 (d, J=2.4 Hz, 2 H, H-Anthracene), 6.81 (dd, J=9.5 Hz, J=4.5 Hz, 2 H, H-Anthracene), 7.12 (d, J=9.5 Hz, 2 H, H-Anthracene), 7.48 (d, J=7.9 Hz, 1 H, H-3), 7.95 (dd, J=8.1 Hz, J=1.9 Hz, 1 H, H-2) 8.13 (d, J=1.9 Hz, 1 H, H-1). +ve electro spray ($C_{30}H_{31}N_3O_6S_2$): expected 593.17; found 594.3 [M+H], 616.2 [M+Na].

0015

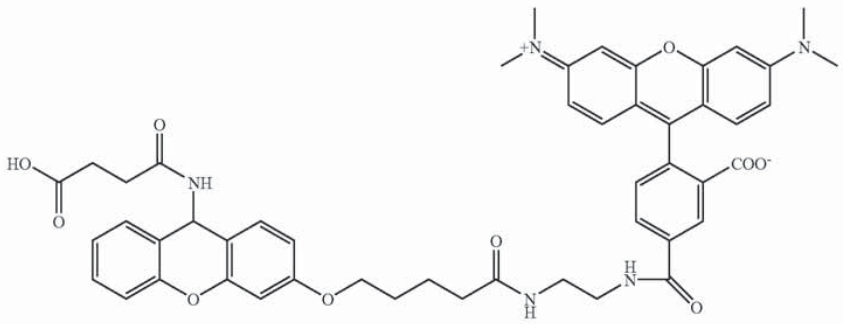


To a solution of 25.8 mg 1*c* (43.4 μmol) in 3 mL DMF (dry) were added 9.9 mg N-hydroxy succinimide (86.8 μmol) and 9.7 mg DCC (47.1 μmol). The mixture was stirred in the dark for 5 h at room temperature and then put in the fridge overnight. The mixture was filtered through a plug of cotton wool in a new flask and to this was added a solution of 865 μl propargylamino dUTP (14.7 μmol, 17 μmol in 1 mL H_2O) and 3 mL sodium borate buffer (0.1 M solution, pH 9). The mixture was stirred overnight. After removal of solvents the residue was dissolved in as little water as possible and purified by HPLC. A Zorbax C18 column was used with 0.1 M triethyl ammonium bicarbonate (TEAB) and acetonitrile as buffers. ^{31}P NMR (400 MHz, D_2O): δ=−4.73 (d), −9.93 (d), 19.03 (t). −ve electro spray ($C_{42}H_{47}N_6O_{19}P_3S_2$ assuming 4 H^+ counter ions): expected 1096.16; found 1092.9. UV in Water: $\lambda_{(max)}$=555 nm $A_{(555)}$ =0.885 (c=0.036 μmol).

Triphosphate (1) was successfully incorporated using Klenow DNA polymerase. The reaction was performed in the following conditions: 50 mM Tris.HCl (pH 7.5), 10 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$, 2 μM compound 3, 100 nM DNA template (previously labelled with P32 and T4 polynucleotide kinase) and 10 units of commercial exo-Klenow (Amersham Corp., Arlington Heights, Ill., USA). The DNA templates were self-complementary hairpins (5'-TACCgTCgACgTCgACgCTggCg-AgCgTgCTgCggTTTTT(C6-amino)TTACCgCAg-CACgCTCgCCAgCg; SEQ ID NO:1). The reaction was performed in 100 μL volume at 37° C. with timepoints taken at 0, 1, 3, 5 and 10 min. The reaction products were electrophoresed down a denaturing (8 M urea) 20% polyacrylamide gel and imaged on a typhoon phosphorimager. Complete single base extension was seen in 1 minute indicating efficient polymerase incorporation (disulfide linker gel, FIG. **3**). A second set of lanes is shown in which the material is exposed to DTT after the incorporation. A different band shift can be seen which shows removal of the dye from the DNA construct, thus a cycle of polymerase incorporation and cleavage has been shown using this disulfide compound.

Example 2

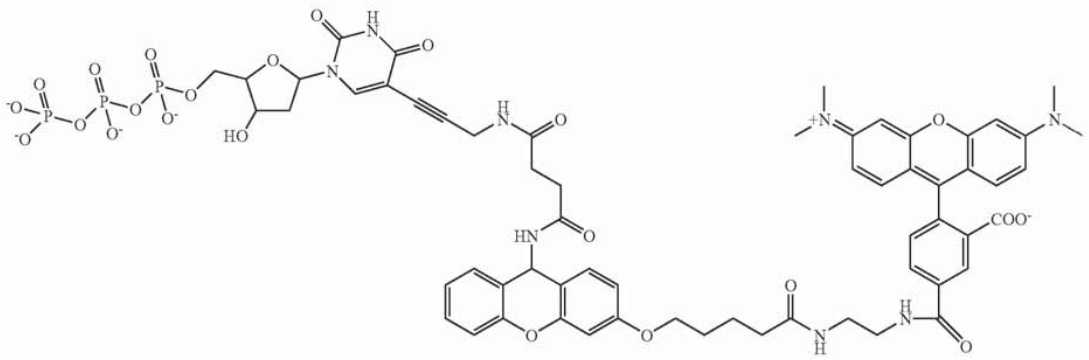
Synthesis of TMR-Sieber Linker Free Acid



5-[-9-[9-(fluorenyl-methyloxycarbonyl)amino]xanthen-3-yl]valeric acid, (42.8 mg, 80 μmol) was stirred at room temperature with disuccinimidyl carbonate (22.5 mg, 88 μmol) and N,N-dimethyl aminopyridine (10.8 mg, 88 μmol) in DMF. After 5 minutes, mono-5-carboxy TMR ethylene diamine (198.9 mg, 40 μmol) was added followed by DIPEA (13.9 μl, 80 μmol). The reaction was stirred at room temperature. After 2 hrs, the reaction mixture was diluted with dichloromethane (100 mL) and the resulting solution was extracted with 1 M aqueous potassium dihydrogen phosphate (50 mL). The DCM layer was separated and evaporated under reduced pressure. The residue was purified by a short column chromatography. The fractions eluting with 40% methanol in chloroform were collected and evaporated under reduced pressure. The residue was then dissolved in dry DMF (1 mL) and N-(2-mercaptoethyl)aminomethyl polystyrene (200 mg, 400 μmol) and DBU (12 μl, 80 μmol). After 10 minutes at room temperature, the resins were filtered off and rinsed with dry DMF (1 mL). All the filtrates were combined and then added to a solution of succinic anhydride (80 mg, 800 μmol), DIPEA (139 μl, 800 μmol) and DMAP (9.8 mg, 80 μmol) in DMF (1 mL). The reaction mixture was then stirred at room temperature. After overnight (16 hrs), all the solvents were evaporated under reduced pressure and the residue was purified by a short column chromatography. The title compound eluted with 30% methanol in chloroform was obtained as purple powders (22 mg, overall yield 63%). ^{1}HNMR [D_6-DMSO]: 8.82 (1H, t, J 5.4, ex.), 8.75 (1H, d, J 8.9, ex.), 8.42 (1H, d, J 1.5), 8.20 (1H, dd, J 8.0 and 1.5), 7.95 (1H, t, J 5.9, ex.), 7.34 (1H, d, J 7.3), 7.30–7.27 (2H, m), 7.21 (1H, d, J 8.5), 7.16–7.07 (2H, m), 6.68 (1H, dd, J 8.8 and 2.5), 6.65 (1H, d, J 2.4), 6.49–6.43 (6H, m), 6.18 (1H, d, J 5.6), 3.95 (1H, t, J 5.9), 3.39–3.36 (2H, m), 3.30–3.27 (2H, m), 2.92 (12H, s), 2.37–2.33 (2H, m), 2.14(2H, t, J 7.2) and 1.70–1.62 (4H, m). MS[(ES(+)], m/z 868.5 (MH^{+}).

Example 3

Synthesis of TMR-Sieber Linker-dUTP (3)

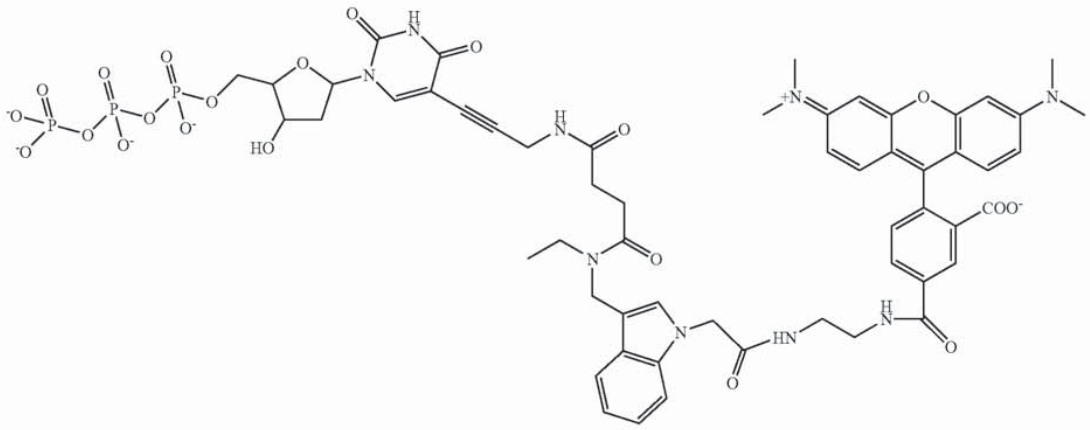


TMR-sieber linker free acid (4.34 mg, 5 μmol) was stirred with disuccinimidyl carbonate (1.74 mg, 7.5 μmol) and N,N-dimethyl aminopyridine (0.92 mg, 7.5 μmol) in DMF (1 mL) at room temperature. After 10 minutes, all the reaction mixture was added to tetra-(tri-butylammonium) salt of 5-(3-aminopropynyl)-2'-deoxyuridine-5'-triphosphate (10 μmol). The reaction was stirred at room temperature for 4 hrs and stored in the fridge overnight. The reaction mixture was then diluted with chilled water (10 mL) and all the resulting solution was applied onto a short column of DEAE A-25. The column was initially eluted with 0.1 M TEAB buffer and then 0.7 M TEAB buffer. The 0.7 M TEAB eluents were collected and evaporated under reduced pressure. The residue was co-evaporated with MeOH (2×10 mL) and then purified by preparative HPLC. The title compound was obtained as triethylammonium salt in 31% yield (based on the quantification of TMR at 555 nm in water (pH 7)). ^{1}HNMR in D_2O indicated two diastereoisomers, due to the sieber linker moiety and there were approximately three triethylammonium count ions. ^{1}HNMR [D_2O]: 8.18 (1H, m), 8.06 (1H, m), 7.76 (0.55H, s), 7.74 (0.45H, s), 7.36–7.09 (5H, m), 6.89–6.72 (3H, m), 6.59–6.37 (5H, m), 6.12 (0.55H, t, J 6.6), 6.05 (0.45H, t, J 6.6), 5.99 (0.45H, d, J 2.5), 5.91 (1.1H, m), 5.88 (0.45H, s), 4.49 (0.55H, m), 4.43 (0.45H, m), 4.00–3.35 (9H, m), 3.30–2.95 (32H, m), 2.65–2.52 (4H, m), 2.25–2.05 (4H, m), 1.62–1.42 (4H, m) and 1.23 (27H, t, J 7.3). ^{31}P [D_2O]: −9.91 ($^{\gamma}$P, d, J 19.2), [−11.08($^{\alpha}$P, d, J 20.1) and −11.30 ($^{\alpha}$P, d, J 20.1), due to two diastereoisomers] and −22.57 ($^{\beta}$P, m). MS[(ES(−)], m/z 1369.1 (M^{-}).

Triphosphate (3) was successfully incorporated using Klenow DNA polymerase. The reaction was performed in the following conditions: 50 mM Tris.HCl (pH 7.5), 10 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$, 2 μM compound 3, 100 nM DNA template (previously labelled with P32 and T4 polynucleotide kinase) and 10 units of commercial exo-Klenow (Amersham Corp. Arlington Heights, Ill., USA). The DNA templates were self-complementary hairpins (5'-TACCgTCgACgTCgACgCTggCgAgCgTgCTgCggTTTTT(C6-amino)TTACCgCAgCACgCTCgCCAgCg; SEQ ID NO:1). The reaction was performed in 100 μL volume at 37° C. with timepoints taken at 0, 1, 3, 5 and 10 min. The reaction products were electrophoresed down a denaturing (8 M urea) 20% polyacrylamide gel and imaged on a typhoon phosphorimager. Complete single base extension was seen in 1 minute indicating efficient polymerase incorporation (Sieber linker gel, FIG. **4**).

Example 4

Synthesis of TMR-Indole Linker-dUTP (4)



Triphosphate (4) was successfully incorporated using Klenow DNA polymerase. The reaction was performed in the following conditions: 50 mM Tris.HCl (pH 7.5), 10 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$, 2 μM compound 3, 100 nM DNA template (previously labelled with P32 and T4 polynucleotide kinase) and 10 units of commercial exo-Klenow (Amersham Corp., Arlington Heights, Ill., USA). The DNA templates were self-complementary hairpins (5'-TACCgTCgACgTCgACgCTggCg-AgCgTgCTgCggTTTTT(C6-amino)TTACCgCAg-CACgCTCgCCAgCg; SEQ ID NO:1). The reaction was performed in 100 μL volume at 37° C. with timepoints taken at 0, 1, 3, 5 and 10 min. The reaction products were electrophoresed down a denaturing (8 M urea) 20% polyacrylamide gel and imaged on a typhoon phosphorimager. Complete single base extension was seen in 1 minute indicating efficient polymerase incorporation (indole linker gel, FIG. **5**).

All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

What is claimed is:

1. A nucleotide or nucleoside molecule, having a base that is linked to a detectable label via a cleavable linker, wherein the molecule has a ribose or deoxyribose sugar moiety comprising a protecting group attached via the 2' or 3' oxygen atom and the cleavable linker and the protecting group are cleavable under identical conditions.

2. The molecule of claim **1**, wherein the base is a purine, or a pyrimidine.

3. The molecule of claim **2**, wherein the base is a deazapurine.

4. The molecule of claim **1**, that is a deoxyribonucleotide triphosphate.

5. The molecule of claim **1**, wherein the detectable label is a fluorophore.

6. The molecule of claim **1**, wherein the linker is acid labile, photolabile or contains a disulphide linkage.

7. A kit, comprising:

(a) individual nucleotides, wherein each nucleotide has a base that is linked to a detectable label via a cleavable linker, and wherein the detectable label linked to each nucleotide can be distinguished upon detection from the detectable label used for other three nucleotides, and each of said individual nucleotides has a deoxyribose sugar moiety comprising a protecting group attached via the 2' or 3' oxygen atom and the cleavable linker and the protecting group are cleavable under identical conditions; and

(b) packaging materials therefor.

8. The kit of claim **7**, further comprising an enzyme and buffers appropriate for the action of the enzyme.

* * * * *

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William R. Zimmerman
Name of Counsel

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The brief contains 13,705 words, excluding the parts of the brief exempted by Federal Rule of Appellate Procedure 32(a)(7)(B)(iii).

2. This brief complies with the typeface requirements of Federal Rule of Appellate Procedure 32(a)(5) and the type style requirements of Federal Rule of Appellate Procedure 32(a)(6).

This brief has been prepared in a proportionally spaced typeface using Microsoft Word in 14-point Times New Roman.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: March 10, 2015

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